

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
7 December 2000 (07.12.2000)

PCT

(10) International Publication Number
WO 00/73498 A1

(51) International Patent Classification⁷: C12Q 1/66,
C07H 19/00, 21/00, 21/02, 21/04

(74) Agents: CORUZZI, Laura, A. et al.; Pennie & Edmonds
LLP, 1155 Avenue of the Americas, New York, NY 10036
(US).

(21) International Application Number: PCT/US00/14986

(22) International Filing Date: 31 May 2000 (31.05.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/324,986 2 June 1999 (02.06.1999) US

(71) Applicant: MILLENNIUM PHARMACEUTICALS,
INC. [US/US]; 75 Sidney Street, Cambridge, MA 02139
(US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE,
DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU,
ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,
LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ,
PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT,
TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— With international search report.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: COMPOSITIONS AND METHODS FOR THE TREATMENT AND DIAGNOSIS OF IMMUNE DISORDERS

WO 00/73498 A1

(57) Abstract: The present invention relates to methods and compositions for the treatment and diagnosis of immune disorders, especially T helper lymphocyte-related disorders, and also for the treatment of mast cell-related processes and disorders, ischemic disorders and injuries, including ischemic renal disorders and injuries. For example, genes which are differentially expressed within and among T helper (TH) cells and TH cell subpopulations, which include, but are not limited to TH0, TH1 and TH2 cell subpopulations are identified. Genes are also identified via the ability of their gene products to interact with gene products involved in the differentiation, maintenance and effector function of such TH cells and TH cell subpopulations. The genes identified can be used diagnostically or as targets for therapeutic intervention. In this regard, the present invention provides methods for the identification and therapeutic use of compounds as treatments of immune disorders, especially TH cell subpopulation-related disorders. Additionally, methods are provided for the diagnostic evaluation and prognosis of TH cell subpopulation-related disorders, for the identification of subjects exhibiting a predisposition to such conditions, for monitoring patients undergoing clinical evaluation for the treatment of such disorders, and for monitoring the efficacy of compounds used in clinical trials. Methods are also provided for the treatment of symptoms associated with mast cell-related processes or disorders and ischemic disorders and injuries using the genes, gene products and antibodies of the invention.

COMPOSITIONS AND METHODS FOR THE TREATMENT
AND DIAGNOSIS OF IMMUNE DISORDERS

5

This is a continuation-in-part of U.S. patent application Serial No. 09/032,337, filed February 27, 1998, which is a continuation-in-part of U.S. patent application Serial No. 08/609,583, filed March 1, 1996, which is a
10 continuation-in-part of U.S. patent application Serial No. 08/487,748, filed June 7, 1995, now U.S. Patent No. 5,721,351, which is a continuation-in-part of U.S. patent application Serial No. 08/398,633, filed March 3, 1995, each of which is incorporated herein by reference in its entirety.

15

1. INTRODUCTION

The present invention relates to methods and compositions for the treatment and diagnosis of immune disorders, especially T lymphocyte-related disorders,
20 including, but not limited to, chronic inflammatory diseases and disorders, such as Crohn's disease, reactive arthritis, including Lyme disease, insulin-dependent diabetes, organ-specific autoimmunity, including multiple sclerosis, Hashimoto's thyroiditis and Grave's disease, contact
25 dermatitis, psoriasis, graft rejection, graft versus host disease, sarcoidosis, atopic conditions, such as asthma and allergy, including allergic rhinitis, gastrointestinal allergies, including food allergies, eosinophilia, conjunctivitis, glomerular nephritis, certain pathogen
30 susceptibilities such as helminthic (e.g., leishmaniasis) and certain viral infections, including HIV, and bacterial infections, including tuberculosis and lepromatous leprosy. For example, genes which are differentially expressed within and among T helper (TH) cells and TH cell subpopulations,
35 which include, but are not limited to TH0, TH1 and TH2 cell subpopulations are identified. Genes are also identified via

the ability of their gene products to interact with gene products involved in the differentiation, maintenance and effector function of such TH cells and TH cell subpopulations. Among the genes identified are ones involved in repair or recovery of tissue from ischemic disorders or injuries. The genes identified can be used diagnostically or as targets for therapeutic intervention. In this regard, the present invention provides methods for the identification and therapeutic use of compounds as treatments of immune disorders, especially TH cell subpopulation-related disorders. The present invention also provides methods for treating ischemic disorders or injuries. Additionally, methods are provided for the diagnostic evaluation and prognosis of TH cell subpopulation-related disorders, for the identification of subjects exhibiting a predisposition to such conditions, for monitoring patients undergoing clinical evaluation for the treatment of such disorders, and for monitoring the efficacy of compounds used in clinical trials.

2. BACKGROUND OF THE INVENTION

Two distinct types of T lymphocytes are recognized: CD8⁺ cytotoxic T lymphocytes (CTLs) and CD4⁺ helper T lymphocytes (TH cells). CTLs recognize and kill cells which display foreign antigens of their surfaces. CTL precursors display T cell receptors that recognize processed peptides derived from foreign proteins, in conjunction with class I MHC molecules, on other cell surfaces. This recognition process triggers the activation, maturation and proliferation of the precursor CTLs, resulting in CTL clones capable of destroying the cells exhibiting the antigens recognized as foreign.

TH cells are involved in both humoral and cell-mediated forms of effector immune responses. With respect to the humoral, or antibody, immune response, antibodies are produced by B lymphocytes through interactions with TH cells. Specifically, extracellular antigens are endocytosed by antigen-presenting cells (APCs), processed, and presented

preferentially in association with class II major histocompatibility complex (MHC) molecules to CD4⁺ class II MHC-restricted TH cells. These TH cells in turn activate B lymphocytes, resulting in antibody production.

5 The cell-mediated, or cellular, immune response, functions to neutralize microbes which inhabit intracellular locations. Foreign antigens, such as, for example, viral antigens, are synthesized within infected cells and presented on the surfaces of such cells in association with class I MHC
10 molecules. This, then, leads to the stimulation of the CD8⁺ class I MHC-restricted CTLs.

Some agents, such as mycobacteria, which cause tuberculosis and leprosy, are engulfed by macrophages and processed in vacuoles containing proteolytic enzymes and
15 other toxic substances. While these macrophage components are capable of killing and digesting most microbes, agents such as mycobacteria survive and multiply. The agents' antigens are processed, though, by the macrophages and presented preferentially in association with class II MHC
20 molecules to CD4⁺ class II MHC-restricted TH cells, which become stimulated to secrete interferon- γ , which, in turn, activates macrophages. Such activation results in the cells' exhibiting increased bacteriocidal ability.

TH cells are composed of at least two distinct
25 subpopulations, termed TH1 and TH2 cell subpopulations. Evidence suggests that TH1 and TH2 subtypes represent extremely polarized populations of TH cells. While such subpopulations were originally discovered in murine systems (reviewed in Mosmann, T.R. and Coffman, R.L., 1989, Ann. Rev.
30 Immunol. 7:145), the existence of TH1- and TH2-like subpopulations has also been established in humans (Del Prete, A.F. et al., 1991, J. Clin. Invest. 88:346; Wiernenga, E.A. et al., 1990, J. Imm. 144:4651; Yamamura, M. et al., 1991, Science 254:277; Robinson, D. et al., 1993, J. Allergy
35 Clin. Imm. 92:313). While TH1-like and TH2-like cells can represent the most extremely polarized TH cell subpopulations, other TH cell subpopulations, such as TH0

cells (Firestein, G.S. et al., 1989, J. Imm. 143:518), which represent TH cells which have characteristics of TH1 and TH2 cell subpopulations.

TH1-like and TH2-like cells appear to function as part
5 of the different effector functions of the immune system (Mosmann, T.R. and Coffmann, R.L., 1989, Ann. Rev. Imm. 7:145). Specifically, TH1-like cells direct the development of cell-mediated immunity, triggering phagocyte-mediated host defenses, and are associated with delayed hypersensitivity.
10 Accordingly, infections with intracellular microbes tend to induce TH1-type responses. TH2 cells drive humoral immune responses, which are associated with, for example, defenses against certain helminthic parasites, and are involved in antibody and allergic responses.

15 It has been noted that the ability of the different TH cell types to drive different immune effector responses is due to the exclusive combinations of cytokines which are expressed within a particular TH cell subpopulation. For example, TH1 cells are known to secrete interleukin-2 (IL-2),
20 interferon- γ (IFN- γ), and lymphotoxin, while TH2 cells secrete interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-10 (IL-10).

It is thought that TH1 and TH2 subpopulations arise from a common naive precursor (referred to as THP). For example,
25 naive CD4⁺ cells from mice which express a single transgenic T cell receptor can be induced to develop into either the TH1 or TH2 cell type. The conditions of antigen stimulation, including the nature and amount of antigen involved, the type of antigen-presenting cells, and the type of hormone and
30 cytokine molecules present seem to all represent determinants of the pattern of TH1 versus TH2 differentiation, with, perhaps, the decisive role belonging to the cytokines present. With such a complex series of possible determinants, a full accounting of the exact factors
35 important in driving TH1 or TH2 differentiation are, as yet largely unknown.

Further, it has recently been noted that, in addition to CD4⁺ TH cells, CD8⁺ CTLs can, under certain conditions, also exhibit TH1-like or TH2-like cytokine profiles (Seder, R.A. et al., 1995, J. Exp. Med. 181:5-7; Manetti, R. et al., 1994, 5 J. Exp. Med. 180:2407-2411; Maggi, E. et al., 1994, J. Exp. Med. 180:489-495). While the precise functional role of such CD8⁺ TH-like cells is currently unknown, these cell subpopulations appear to have great relevance to immune responses against infectious agents such as viruses and 10 intracellular parasites.

Once TH1 and TH2 subpopulations are expanded, the cell types tend to negatively regulate one another through the actions of cytokines unique to each. For example, TH1-produced IFN- γ negatively regulates TH2 cells, while TH2- 15 produced IL-10 negatively regulates TH1 cells. Moreover, cytokines produced by TH1 and TH2 antagonize the effector functions of one another (Mosmann, T.R. and Moore, 1991, Immunol. Today 12:49).

Failure to control or resolve an infectious process 20 often results from an inappropriate, rather than an insufficient immune response, and can underlie a variety of distinct immunological disorders. Such disorders can include, for example, atopic conditions (i.e., IgE-mediated allergic conditions) such as asthma, allergy, including 25 allergic rhinitis, dermatitis, including psoriasis, pathogen susceptibilities, chronic inflammatory disease, organ-specific autoimmunity, graft rejection and graft versus host disease. For example, nonhealing forms of human and murine leishmaniasis result from strong but counterproductive TH2- 30 like-dominated immune responses. Lepromatous leprosy also appears to feature a prevalent, but inappropriate, TH2-like response.

It is possible that another example can be HIV infection. Here, it has been suggested that a drop in the 35 ratio of TH1-like cells to other TH cell subpopulations can play a critical role in the progression toward disease symptoms. Further, it has been noted that, at least in

vitro, TH2-like clones appear to be more efficient supporters of HIV viral replication than TH1-like clones.

Further, while TH1-mediated inflammatory responses to many pathogenic microorganisms are beneficial, such responses to self antigens are usually deleterious. It has been suggested that the preferential activation of TH1-like responses is central to the pathogenesis of such human inflammatory autoimmune diseases as multiple sclerosis and insulin-dependent diabetes. For example, TH1-type cytokines predominate in the cerebrospinal fluid of patients with multiple sclerosis, pancreases of insulin-dependent diabetes patients, thyroid glands of Hashimoto's thyroiditis, and gut of Crohn's disease patients, suggesting that such patients mount a TH1-like, not a TH2-like, response to the antigen(s) involved in the etiopathogenesis of such disorders.

A primary goal, for both diagnostic and therapeutic reasons, therefore, would be the ability to identify, isolate and/or target members of a particular TH cell subpopulation. The ability to identify those genes which are differentially expressed within and/or among such TH cell subpopulations is required to achieve such a goal. To date, investigations have focused on the expression of a limited number of specific known cytokines and cytokine receptors in the TH cell population. Cytokines, however, exert effects on cell types in addition to specific TH cell subpopulations, *i.e.*, exhibit a variety of pleiotropic effects. It would be beneficial, therefore, to identify reliable markers (*e.g.*, gene sequences) of TH cell subpopulations whose effects are TH cell subpopulation specific, *e.g.*, which, unlike secreted cytokines, are TH cell subpopulation specific.

3. SUMMARY OF THE INVENTION

The present invention relates to methods and compositions for the treatment of immune disorders, especially T helper (TH) cell and TH cell-like related disorders. The present invention additionally relates to methods and compositions for treating, ameliorating or

modulating ischemic disorders or injuries or mast cell-related processes or disorders.

First, genes are identified and described which are differentially expressed within and among TH cells and TH cell subpopulations. Second, genes are identified and described which are differentially expressed within TH cell subpopulations in TH cell subpopulation-related disorders. The modulation of the expression of the identified genes and/or the activity of the identified gene products can be utilized therapeutically to ameliorate immune disorder symptoms and to modulate TH cell responsiveness, for example, responsiveness to antigen. Further, the identified genes and/or gene products can be used to diagnose individuals exhibiting or predisposed to such immune disorders. Still further, the identified genes and/or gene products can be used to detect TH cell responsiveness, for example, responsiveness to antigen.

"Differential expression," as used herein, refers to both quantitative as well as qualitative differences in the genes' temporal and/or cellular expression patterns within and among the TH cell subpopulations. Differentially expressed genes can represent "fingerprint genes" and/or "target genes".

"Fingerprint gene," as used herein, refers to a differentially expressed gene whose expression pattern can be utilized as part of a prognostic or diagnostic evaluation of immune disorders, e.g., TH cell-related disorders, or which, alternatively, can be used in methods for identifying compounds useful in the treatment of such disorders. For example, the effect of the compound on the fingerprint gene expression normally displayed in connection with the disorder can be used to evaluate the efficacy of the compound as a treatment for such a disorder, or may, additionally, be used to monitor patients undergoing clinical evaluation for the treatment of such disorders.

"Fingerprint pattern," as used herein, refers to the pattern generated when the expression pattern of a series

(which can range from two up to all the fingerprint genes which exist for a given state) of fingerprint genes is determined. A fingerprint pattern can be used in the same diagnostic, prognostic, and compound identification methods
5 as the expression of a single fingerprint gene.

"Target gene," as used herein, refers to a differentially expressed gene involved in immune disorders, e.g., TH cell related disorders, such that modulation of the level of target gene expression or of a target gene product
10 activity can act to ameliorate the immune disorder.

Compounds that modulate target gene expression or activity of the target gene product can be used in the treatment of immune disorders.

Further, "pathway genes" are defined via the ability of
15 their gene products to interact with gene products involved in TH cell subpopulation-related disorders and/or to interact with gene products which are involved in the differentiation and effector function of the TH cell subpopulations. Pathway genes can also exhibit target gene and/or fingerprint gene
20 characteristics.

Although the target, fingerprint and/or pathway genes described herein can be differentially expressed within and/or among TH cell subpopulations, and/or can interact with TH cell subpopulation gene products, the genes can also be
25 involved in mechanisms important to additional immune processes.

The invention encompasses the following nucleotides, host cells expressing such nucleotides and the expression products of such nucleotides: (a) nucleotides that encode a
30 mammalian differentially expressed and/or pathway gene product including, but not limited to a human and murine 10, 54, 57, 105, 106, 161 and 200 gene product; (b) nucleotides that encode portions of a differentially expressed and/or pathway gene product that corresponds to its functional
35 domains, and the polypeptide products encoded by such nucleotide sequences, and in which, in the case of receptor-type gene products, such domains include, but are not limited

to extracellular domains (ECD), transmembrane domains (TM) and cytoplasmic domains (CD); (c) nucleotides that encode mutants of a differentially expressed and/or pathway gene product, in which all or part of one of its domains is
5 deleted or altered, and which, in the case of receptor-type gene products, such mutants include, but are not limited to, soluble receptors in which all or a portion of the TM is deleted, and nonfunctional receptors in which all or a portion of CD is deleted; and (d) nucleotides that encode
10 fusion proteins containing a differentially expressed and/or pathway gene product or one of its domains fused to another polypeptide.

The present invention also includes the products of such fingerprint, target, and pathway genes, as well as antibodies
15 to such gene products. Furthermore, the engineering and use of cell- and animal-based models of TH cell subpopulation-related disorders to which such gene products can contribute, are also described.

The present invention also relates to methods for
20 prognostic and diagnostic evaluation of various TH cell subpopulation-related disorders, and for the identification of subjects who are predisposed to such disorders. Furthermore, the invention provides methods for evaluating the efficacy of drugs for immune disorders, and monitoring
25 the progress of patients involved in clinical trials for the treatment of such disorders.

The TH cell subpopulation-related disorders described herein can include, for example, TH1 or TH1-like related disorders or can, alternatively, include TH2 or TH2-like
30 related disorders. Examples of TH1 or TH1-like related disorders include chronic inflammatory diseases and disorders, such as Crohn's disease, reactive arthritis, including Lyme disease, insulin-dependent diabetes, organ-specific autoimmunity, including multiple sclerosis,
35 Hashimoto's thyroiditis and Grave's disease, contact dermatitis, psoriasis, graft rejection, graft versus host disease and sarcoidosis. Examples of TH2 or TH2-like related

disorders include atopic conditions, such as asthma and allergy, including allergic rhinitis, gastrointestinal allergies, including food allergies, eosinophilia, conjunctivitis, glomerular nephritis, certain pathogen
5 susceptibilities such as helminthic (e.g., leishmaniasis) and certain viral infections, including HIV, and bacterial infections, including tuberculosis and lepromatous leprosy.

- Ischemic disorder or injury can be treated via the methods of the invention. "Ischemic disorder or injury"
10 refers to any disorder or injury to tissues or organs which results from local deficiency of the blood supply and/or of said tissue or organs. Such deficiencies of the blood supply and/or hypoxia are generally produced by a restriction or obstruction of the blood supply to said tissue or organs.
- 15 Ischemic disorders or injuries which may be treated by the methods of the present invention include, but are by no means limited to, ischemic renal disease or injury, or myocardial ischemia such as angina pectoris. Ischemic disorders or injuries which may be treated by the methods of the present
20 invention also include damage or injury to tissue or organs due to an infarction, such as damage to the heart, brain (such as in a stroke), spleen, kidney, intestine, lung, and testes. The methods of the invention may also be used to regulate the extent or degree of ischemic injury in other
25 tissues, such as tumor tissues including, but not limited to, tumors of the uterus and ovaries. The ischemic disorders or injuries which may be treated by the methods of the present invention still further include ischemic injury or damage to transplanted organs which occurs during transplant.
- 30 It is further contemplated that the methods and compositions described herein can be utilized in the prognostic and diagnostic evaluation of disorders involving other immune cells, including CD8⁺ CTLs, exhibiting TH-like cell subpopulation gene expression patterns and/or activity.
- 35 It is still further contemplated that the methods and compositions described herein can be utilized in the amelioration of symptoms stemming from disorders involving

such immune cells, especially such CD8⁺ CTLs, which exhibit TH-like cell subpopulation gene expression patterns and/or activity.

The invention further provides methods for the
5 identification of compounds which modulate the expression of genes or the activity, e.g. level, of gene products involved in TH cell subpopulation-related disorders and processes relevant to the differentiation, maintenance and/or effector function of the subpopulations. For example, presented
10 herein are methods for identifying compounds which affect the level of 103 gene expression and/or gene product.

In addition, the present invention provides methods for identifying compounds which bind to gene products of the differentially expressed sequences identified herein. For
15 example, such methods include, but are not limited to, methods for identifying compounds which bind to a 103 gene product.

Still further, the present invention provides methods for the treatment of TH cell subpopulation-related disorders
20 which can, for example, involve the administration of such modulatory compounds to individuals exhibiting TH cell subpopulation-related disorder symptoms or tendencies. Additionally, treatment can result in the stimulation or depletion of one or more of the TH cell subpopulations.

25 "Stimulation", as used herein, can refer to an effective increase in the number of cells belonging to a TH cell subpopulation, via, for example, the proliferation of such TH cell subpopulation cells. The term can also refer to an increase in the activity of cells belonging to a TH cell
30 subpopulation, as would be evidenced, for example, by a per cell increase in the expression of the TH cell subpopulation-specific cytokine pattern.

"Depletion", as used herein, can refer to an effective reduction in the number of cells belonging to a TH cell
35 subpopulation, via, for example, a reduction in the proliferation of such TH cell subpopulation cells. The term can also refer to a decrease in the activity of cells

belonging to a TH cell subpopulation, as would be evidenced, for example, by a per cell decrease in the expression of the TH cell subpopulation-specific cytokine pattern.

In an alternative embodiment of the present invention, 5 the methods and compositions described herein can also be used in the treatment of ischemic disorders or injuries. For example, presented herein are methods of using the 200 gene, its gene product, and antibodies thereto to treat or regulate ischemic disorders and/or injuries. In particular, the genes 10 or gene products of the invention may be administered to an individual so as to ameliorate the symptoms of the ischemic disorder or injury. Further, compounds, such as specific antibodies, including monoclonal antibodies, which bind specifically to the genes or gene products of the present 15 invention and modulate their expression or activity, may also be administered to an individual suffering from an ischemic disorder or injury.

The invention is based, in part on systematic search strategies involving paradigms which utilize TH0, TH1, TH2, 20 TH1-like and TH2-like cells, in systems which mimic the activity of the immune system or immune disorders, coupled with sensitive and high-throughput gene expression assays, to identify genes differentially expressed within and/or among TH cell subpopulations. In contrast to approaches that 25 merely evaluate the expression of a single known gene product presumed to play a role in some immune cell-related process or disorder, the search strategies and assays used herein permit the identification of all genes, whether known or novel, which are differentially expressed within and among TH 30 cell subpopulations, as well as making possible the characterization of their temporal regulation and function in the TH cell response and/or in TH cell mediated disorders. This comprehensive approach and evaluation permits the discovery of novel genes and gene products, as well as the 35 identification of a constellation of genes and gene products (whether novel or known) involved in novel pathways (e.g., modulation pathways) that play a major role in the TH-cell

mediated immune responses and TH cell subpopulation-related disorders. Thus, the present invention makes possible the identification and characterization of targets useful for prognosis, diagnosis, monitoring, rational drug design,
5 and/or therapeutic intervention of immune system disorders.

The Examples described in Sections 6 through 8, below, demonstrate the successful use of the search strategies of the invention to identify genes which are differentially expressed among and/or within TH cell subpopulations.

10 Section 9 describes the successful cloning of a human homolog of one of the identified genes (the 200 gene).

The 102 and 103 genes represent genes which, while previously known, are shown here to be differentially expressed among TH cell subpopulations. Specifically, the
15 102 gene corresponds to the Granzyme A, or Hanukah factor, gene, which encodes a trypsin-like serine protease. While this gene had previously been reported to be expressed in natural killer cells and a fraction of CD4⁺ cells, the results described herein reveal, for the first time, that the
20 gene is differentially expressed within the TH2 cell subpopulation. Specifically, the 102 gene is expressed at a level many-fold higher in the TH2 cell subpopulation than in the TH1 cell subpopulation.

The 103 gene corresponds to a gene known as the T1, ST-2
25 or Fit-1 gene, which encodes, possibly via alternative splicing, both transmembrane and soluble gene products. The gene 103 products belong to the immunoglobulin superfamily, and bear a high resemblance to the interleukin-1 (IL-1) receptor. The results presented herein demonstrate, for the
30 first time, that this gene is expressed, in vivo, in a tightly controlled TH2-specific fashion. Thus, given its status as both a TH2 cell subpopulation-specific marker and a cell surface protein, the gene 103 products can be utilized in a variety of methods to diagnose and/or modulate immune
35 system disorders, in particular TH2 cell subpopulation-related disorders. Further, results, including results obtained in vivo in an animal model for asthma, a TH2-like

disorder, are presented herein which indicate that the 103 gene product provides a critical signal to TH2 effector cells.

In addition to these known genes, the systematic search 5 strategies described herein were used to identify several novel genes which are differentially expressed within and/or among TH cell subpopulations. Specifically, these include the 10, 54, 57, 105, 106, 161 and 200 genes.

The 54, 105, 106 and murine 200 genes are each shown to 10 be differentially expressed within the TH1 cell subpopulation. Specifically, these genes are expressed at levels many-fold higher in TH1 cell subpopulations than in TH2 cell subpopulations.

The novel 54 gene product is a 371 amino acid cysteine 15 protease, as evidenced by the presence of three thiol protease domains at approximately amino acid residue 145 to 156 (CYS domain), approximately amino acid residue 287 to 297 (HIS domain) and approximately amino acid residue 321 to 340 (ASN domain) of the 54 gene product amino acid sequence.

20 The 10 and 57 genes represent TH inducible gene sequences. That is, the expression of such genes in unstimulated TH cells is either undetectable or barely detectable, but is significantly upregulated in both stimulated TH1 and stimulated TH2 cells. Thus, the 10 and 57 25 genes and/or their gene products can represent new targets for therapeutic treatment as part of a non-TH cell subpopulation dependent intervention program.

The 10 gene product is a 338 amino acid receptor molecule which is a particularly suitable target for such a 30 program in that the 10 gene product belongs to a class of proteins having a seven transmembrane domain sequence motif, which tend to represent G protein-coupled receptor molecules. The 10 gene product structure, therefore, indicates that it may be involved in signal transduction events which may be 35 important to T cell responses in general, and further indicates that modulation of 10 gene product may effectively ameliorate a wide range of T cell-related disorders.

Specifically, because the 10 gene product is a transmembrane product, its activity, via either a physical change in the number of 10 gene-expressing cells or by a change in the functional level of 10 gene product activity, 5 can be particularly amenable to modulation. For example, natural ligands, derivatives of natural ligands and antibodies which bind to the 10 gene product can be utilized to reduce the number of induced T cells present by either physically separating such cells away from other cells in a 10 population, or, alternatively, by targeting the specific destruction of the induced T cells or inhibiting the proliferation of such T cells.

Additionally, compounds such as 10 gene sequences or gene products such as, for example, soluble 10 gene products, 15 can be utilized to reduce the level of induced T cell activity, and, ultimately, bring about the amelioration of a wide range of T cell-related disorders. For example, in the case of soluble gene 10 gene products, the compounds can compete with the endogenous (i.e., natural) ligand for the 10 20 gene product, leading to a modulation of induced T cell activity. Soluble proteins or peptides, such as peptides comprising one or more of the extracellular domains, or portions and/or analogs thereof, of the 10 gene product, including, for example, soluble fusion proteins such as Ig- 25 tailed fusion proteins, can be particularly useful for this purpose. Additionally, antibodies directed against one or more of the extracellular portions of the 10 gene product may either reduce 10 gene product function by, for example, blocking ligand binding. Additionally, antibodies directed 30 against the 10 gene product can, in certain instances, serve to increase the level of 10 gene product activity.

The receptor nature of the 10 gene product makes possible useful methods for the identification of compounds which modulate the receptor's functional activity and which 35 can act as therapeutic agents in the amelioration of a wide range of T cell-related disorders. For example, functional assays which measure intracellular calcium release levels may

be utilized to identify compounds which act as either agonists or antagonists of 10 gene product activity. Such assays may, additionally, be utilized to identify the natural 10 gene product ligand. Still further, any of these

5 modulatory compounds can be utilized as therapeutic agents for the amelioration of a wide range of T cell-related disorders.

Finally, the 161 gene is shown to be an additional new and potentially interesting target for a therapeutic method
10 aimed at the amelioration of immune disorder related symptoms. In fact, it is possible that 161 gene expression may be indicative of the presence of yet another TH cell subpopulation, in addition to TH1, TH2 and TH0 cell subpopulations.

15 The identification of TH cell subpopulation specific markers can be utilized in the treatment of a number of immune disorders, especially TH cell subpopulation-related disorders. For example, markers for the TH2 subpopulation can be used to ameliorate conditions involving an
20 inappropriate IgE immune response, including but not limited to the symptoms which accompany atopic conditions such as allergy and/or asthma. IgE-type antibodies are produced by stimulated B cells which require, at least in part, IL-4 produced by the TH2 cell subpopulation. Therefore, a
25 treatment which reduces the effective concentration of secreted IL-4, e.g., by reducing the activity or number of TH2 cells, will bring about a reduction in the level of circulating IgE, leading, in turn, to the amelioration or elimination of atopic conditions. Any of the TH2-specific
30 gene products described herein can, therefore, be used as a target to reduce or deplete the number and/or activity of TH2 cell subpopulation cells for the treatment of such conditions.

The 103 gene can be particularly suitable for this
35 purpose since one of its gene products is a membrane-bound TH2 cell subpopulation molecule. Accordingly, natural ligands, derivatives of natural ligands and antibodies which

bind to this 103 gene product, can be utilized to reduce the number of TH2 cells present by either physically separating such cells away from other cells in a population, or, alternatively, by targeting the specific destruction of TH2
5 cells or inhibiting the proliferation of such TH2 cells. Additionally, compounds such as 103 gene sequences or gene products can be utilized to reduce the level of TH2 cell activity, cause a reduction in IL-4 production, and, ultimately, bring about the amelioration of IgE and/or TH2-
10 related disorders. For example, the compounds can compete with the endogenous (*i.e.*, natural) ligand for the 103 gene product. The resulting reduction in the amount of ligand-bound 103 gene transmembrane protein will modulate TH2 cellular activity. Soluble proteins or peptides, such as
15 peptides comprising the extracellular domain, the secreted form, or portions and/or analogs thereof, of the 103 gene product, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins, can be particularly useful for this purpose. In certain instances, antibodies directed
20 against the 103 gene product, such as directed against the extracellular domain of the 103 gene product, can be utilized for this purpose.

The identification of TH cell subpopulation specific markers can additionally be utilized in the treatment of a
25 TH1 cell subpopulation-related disorders. For example, markers for the TH1 cell subpopulation can be used to ameliorate conditions involving an inappropriate cell-mediated immune response, including, but not limited to chronic inflammatory and autoimmune disorders. Further,
30 transgenic animals overexpressing or misexpressing such gene sequences and/or transgenic "knockout" animals exhibiting little or no expression of such sequences can be utilized as animal models for TH cell subpopulation-related disorders. The Example presented in Section 11, below, describes the
35 production of 200 and 103 transgenic animals.

TH1 cell subpopulation specific gene sequences and/or gene products such as the 54 (which encodes a 371 amino acid

cysteine protease gene product), 105, 106 and 200 (the murine homolog of which encodes a 280 amino acid transmembrane gene product, the human homolog of which encodes a 301 amino acid transmembrane gene product, both of which are members of the 5 Ig superfamily) genes can, therefore, be suitable for ameliorating such TH1 cell subpopulation-related disorders.

The 200 gene product can be particularly suitable for such a purpose in that it is not only TH1 cell subpopulation-restricted, but the Ig superfamily 200 gene product is, 10 additionally, membrane-bound. Therefore, natural ligands, derivatives of natural ligands and antibodies which bind to the 200 gene product can be utilized to reduce the number of TH1 cells present by either physically separating such cells away from other cells in a population, or, alternatively, by 15 targeting the specific destruction of TH1 cells or inhibiting the proliferation of such TH1 cells. Additionally, compounds such as 200 gene sequences or gene products such as soluble 200 gene products, can be utilized to reduce the level of TH2 cell activity, thus bringing about the amelioration of TH1 20 cell subpopulation-related disorders. For example, the compounds can compete with the endogenous (i.e., natural) ligand for the 200 gene product. The resulting reduction in the amount of ligand-bound 200 gene transmembrane protein will modulate TH2 cellular activity. Soluble proteins or 25 peptides, such as peptides comprising the extracellular domain, or portions (such as, for example, the Ig portion) and/or analogs thereof, of the 200 gene product, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins, can be particularly useful for this purpose. The 30 Example presented in Section 10, below, describes the construction and expression of 200 gene product and 103 gene product Ig fusion constructs and proteins.

Further, the Example presented in Section 12, below, describes successful use of antibodies directed against the 35 103 gene product as well as 103/Ig fusion proteins to ameliorate symptoms of asthma in an accepted animal model for the TH2-related disorder. Thus, the results indicate that

the 103 gene product provides a critical signal to TH2 cells and can successfully be used as a target for selective modulation of TH immune responses (e.g., for selective suppression of TH2 immune responses and/or selective
5 enhancement of TH1 immune responses).

The invention is also based, in part, on the discovery that among the genes and gene products described herein are ones also involved in processes related to tissue repair and remodeling after injury, particularly after ischemic injury.
10 In particular, the example presented in Section 13, below, demonstrates the successful use of antibodies which bind to the extracellular domain of the 200 gene product to inhibit repair of ischemic kidney injury. Thus, the invention also makes possible the treatment of ischemic disorders and
15 injuries. The invention is also based, in part, on the discovery that the 103 gene is expressed in mast cells, as demonstrated in the Example presented in Section 14, below.

3.1. DEFINITIONS

20 The term "TH cell subpopulation", as used herein, refers to a population of TH cells exhibiting a gene expression pattern (e.g., a discrete pattern of cytokines and/or receptor or other cell surface molecules) and activity which are distinct from the expression pattern and activity of
25 other TH cells. Such TH cell subpopulations can include, but are not limited to, TH0, TH1 and TH2 subpopulations, which will, for clarity and example, and not by way of limitation, be frequently used herein as representative TH cell subpopulations.

30 The term "TH-like cell subpopulation" (e.g., "TH1-like" or "TH2-like"), as used herein is intended to refer not only to a population of CD4⁺ TH cells having the properties described, above, for a TH cell subpopulation, but also refers to CD4⁺ cells, including CD8⁺ CTLs, which exhibit TH-
35 like cytokine expression patterns.

"Differential expression", as used herein, refers to both quantitative as well as qualitative differences in the genes' temporal and/or cellular expression patterns.

"Target gene", as used herein, refers to a
5 differentially expressed gene involved in immune disorders and/or in the differentiation, maintenance and/or effector function of TH cell subpopulations, such that modulation of the level of target gene expression or of target gene product presence and/or activity can, for example, act to result in
10 the specific depletion or repression, or, alternatively, the stimulation or augmentation of one or more TH cell subpopulation, bringing about, in turn, the amelioration of symptoms of immune disorders, e.g., TH cell subpopulation-related disorders. A target gene can also exhibit
15 fingerprint and/or pathway gene characteristics.

"Fingerprint gene," as used herein, refers to a differentially expressed gene whose mRNA expression pattern, protein level and/or activity can be utilized as part of a prognostic or diagnostic in the evaluation of immune
20 disorders, e.g., TH cell subpopulation-related disorders, or which, alternatively, can be used in methods for identifying compounds useful for the treatment of such disorders, by, for example, evaluating the effect of the compound on the fingerprint gene expression normally displayed in connection
25 with the disease. A fingerprint gene can also exhibit target and/or pathway gene characteristics.

"Fingerprint pattern," as used herein, refers to the pattern generated when the mRNA expression pattern, protein level and/or activity of a series (which can range from two
30 up to all the fingerprint genes which exist for a given state) of fingerprint genes is determined. A fingerprint pattern can be a part of the same methods described, above, for the expression of a single fingerprint gene.

"Pathway genes", as used herein, refers to a gene whose
35 product exhibits an ability to interact with gene products involved in immune disorders, e.g., TH cell subpopulation-related disorders and/or to interact with gene products which

are involved in the differentiation and effector function of TH cell subpopulations. Pathway genes can also exhibit target gene and/or fingerprint gene characteristics.

"Negative modulation", as used herein, refers to a reduction in the level and/or activity of target gene product relative to the level and/or activity of the target gene product in the absence of the modulatory treatment. Alternatively, the term, as used herein, refers to a reduction in the number and/or activity of cells belonging to the TH cell subpopulation relative to the number and/or activity of the TH cell subpopulation in the absence of the modulatory treatment.

"Positive modulation", as used herein, refers to an increase in the level and/or activity of target gene product relative to the level and/or activity of the gene product in the absence of the modulatory treatment. Alternatively, the term, as used herein, refers to an increase in the number and/or activity of cells belonging to the TH cell subpopulation, relative to the number and/or activity of the TH cell subpopulation in the absence of the modulatory treatment.

"Ischemic disorder or injury", as used herein, refers to any disorder or injury to tissues or organs which results from local deficiency of the blood supply and/or hypoxia to said tissue or organs. Such deficiency of the blood supply and/or hypoxia is generally produced by a restriction or obstruction of the blood supply to said tissue or organs.

The term "mast-cell related processes or disorders", as used herein, includes, but is not limited to, atherosclerosis and myocardial ischemia/reperfusion.

4. DESCRIPTION OF THE FIGURES

FIG. 1. Differential display analysis of RNA from murine TH cell subsets. Splenic T cells derived from T cell receptor transgenic mice were differentiated in vitro to become polarized populations of TH1 or TH2 subtypes. Lane 1: TH2 population 24 hours after tertiary stimulation; lane 2:

TH1 population 24 hours after tertiary stimulation; lane 3:
TH2 population 1 week after secondary stimulation; lane 4:
TH1 population 1 week after secondary stimulation; lane 5:
TA3 cell line, which was used as antigen presenting cell
5 (APC) for in vitro stimulation. (This sample was used as a
negative control.) Each set of lanes consists of duplicates
(a and b), in which cDNAs were independently generated from
the same source of RNA. Arrow points to differentially
expressed sequence, which is referred to herein as band 102.
10 Further, the gene corresponding to band 102 is referred
to herein as the 102 gene. All lanes are products of a
polymerase chain reaction (PCR) in which T₁₁GG was used as the
3' oligonucleotide and a random 10mer oligonucleotide (Oligo
#4, OP-D kit, Operon, Inc.) was used as the 5'
15 oligonucleotide.

FIG. 2. Nucleotide sequence of clone 102.1 of band 102
(SEQ. ID NO: 1). The gene corresponding to band 102 is
referred to herein as the 102 gene.

20

FIG. 3. Northern blot analysis of confirming
differential regulation of the 102 gene within primary
TH1/TH2 cultures and murine tissues. RNA was harvested from
T cell lines derived from a T cell receptor transgenic strain
25 stimulated in vitro. Lane 1, TH2, 40 hours after second
stimulation; lane 2, TH1, 40 hours after second stimulation;
lane 3, TH2 population 24 hours after tertiary stimulation;
lane 4, TH1, 24 hours after tertiary stimulation; lane 5,
murine thymus; lane 6, murine spleen. Five micrograms of
30 total RNA was used per lane. The cloned band 102 sequence
was used as a probe.

FIG. 4A. Nucleotide sequence clone 103.1 of band 103
(SEQ ID NO:2). The gene corresponding to band 103 is
35 referred to herein as gene 103.

FIG. 4B. 103 gene products. This diagram illustrates the relationship between the sequence encoded by band 103, 103 gene (also known as ST-2, T1 and Fit-1) products and the IL-1 receptor polypeptide structure. The extracellular, 5 transmembrane and cytoplasmic domains of the proteins are noted, along with the amino acid residues marking the boundaries of these domains. (Adapted from Yanagisawa et al., 1993, FEBS Lett. 318:83-87.)

10 FIG. 4C. The nucleotide sequence encoding the secreted form of murine 103 gene product is depicted (SEQ ID NO:49; Accession No. E07714).

FIG. 4D. The amino acid sequence of the secreted form 15 of murine 103 is depicted (SEQ ID NO:47; Accession No. P14719).

FIG. 4E. The nucleotide sequence encoding the transmembrane murine 103 gene product is depicted (SEQ ID 20 NO:38; Accession No. E08652).

FIG. 4F. The amino acid sequence of the transmembrane receptor of murine 103 is depicted (SEQ ID NO:39; Accession No. S29498). The extracellular domain of the full length, 25 transmembrane product extends from amino acid residue 1 to 342 of SEQ ID NO: 38 (SEQ ID NO:41), the transmembrane domain of the full length, transmembrane product extends from amino acids 343 to 366 of SEQ ID NO: 38 (SEQ ID NO:48) the intracellular domain of the full length, transmembrane 30 product extends from amino acid residues 367 to 567 of SEQ ID NO: 38 (SEQ ID NO:43).

FIG. 4G. The nucleotide sequence encoding the secreted product of the human 103 gene is depicted (SEQ ID NO:44; 35 Accession No. NM_003856).

FIG. 4H. The amino acid sequence of the secreted product of the human 103 gene is depicted (SEQ ID NO:45; Accession No. NM_003856).

5 FIG. 5. Quantitative RT-PCR analysis of 103 gene expression in polarized populations of murine TH cells. RNA samples were harvested from cultured T cell populations 24 hours after tertiary stimulation with antigen. cDNA samples were PCR amplified and the products of those reactions were
10 electrophoresed on a 1% agarose gel and visualized by ethidium bromide staining. 103 gene expression is shown in the upper panel. γ -actin data, bottom panel, was included as a control for differences in sample quality. The numbers above each lane represent the dilution factors of each cDNA.
15 The same cDNA samples were used for both the 103 gene and the γ -actin amplifications.

FIG. 6. Northern blot analysis of 103 gene expression in representative murine TH cell lines (TH2: CDC25, D10.G4,
20 DAX; TH1: AE7.A, Dorris, D1.1). Clones were either unstimulated (-) or stimulated (+) for 6 hours with plate-bound anti-CD3 antibody. Ten micrograms of total RNA were loaded per lane. The positions of 18s and 28s ribosomal RNA are shown as reference markers.

25

FIG. 7. Northern blot analysis of 103 gene expression in T cell clones and murine tissues. Lane 1: DAX cells, no stimulation; lane 2, AE7 cells, stimulation; lane 3, AE7 cells, no stimulation; lane 4, D10.G4 cells, stimulation;
30 lane 5, D10.G4 cells, no stimulation; lane 6, brain; lane 7, heart; lane 8, lung; lane 9, spleen; lane 10, liver. Clones were stimulated with plate-bound anti-CD3 antibody for 6 hours. 7.5 and 10 micrograms total RNA was used for each cell line and each tissue, respectively. a, b, and c arrows
35 refer to RNA encoding full length (a) and truncated (b,c) forms of the 103 gene. The positions of 18s and 28s ribosomal RNA markers are shown.

FIG. 8. RNase protection analysis of 103 gene mRNA, illustrating regulation of 103 gene expression in murine TH cell clones. Lanes 2-6: β -actin protection; lanes 9-13: 103 gene protection; lanes 1 and 8: markers; lanes 2 and 9: 5 unstimulated TH1 clones; lanes 3 and 10: stimulated TH1 clones; lanes 4 and 11: unstimulated TH2 clones; lanes 5 and 12: stimulated TH2 clones; lanes 6 and 13: fully RNase A digested unprotected probe; lanes 7 and 14: probe alone, in absence of added RNase.

10 Expected fragment sizes:

β -actin protected probe: 250 nucleotides;

β -actin full length probe: 330 nucleotides;

103 gene long form fragment: 257 nucleotides;

103 gene short form fragment: 173 nucleotides;

15 103 gene full length probe: 329 nucleotides.

FIG. 9. The full length 10 gene nucleotide sequence (SEQ ID NO: 3) is shown on the top line, while the derived amino acid sequence of the 10 gene product (SEQ ID NO: 9) is 20 shown on the bottom line. The underlined portion of the nucleotide sequence corresponds to the band 10 nucleotide sequence. The data shown in FIG. 10A-C was obtained through the use of the portion of the 10 gene product which is encoded by the band 10 nucleotide sequence.

25

FIG. 10A-C. 10 gene hydrophilicity data, indicating that the 10 gene-derived amino acid sequence predicts the presence of a seven transmembrane domain structural motif. 10A) platelet activating factor receptor hydrophilicity plot 30 illustrating the protein's seven transmembrane domain structural motif; 10B) 10 gene hydrophilicity plot illustrating a portion of the protein's putative seven transmembrane domain structural motif; 10C) platelet activating factor receptor hydrophilicity plot illustrating 35 part of the protein's seven transmembrane structural motif.

FIG. 11. Chromosomal mapping of locus containing the 10 gene sequence. A map of a portion of mouse chromosome 12 is shown. Numbers to left of chromosome are in centiMorgans; D12NDS11, D12MIT4, and D12MIT8 represent mouse microsatellite 5 markers; TH10 represents 10 gene.

FIG. 12. Nucleotide sequence of clone 7 of band 57 (SEQ ID NO:4). The gene corresponding to band 57 is referred to herein as the 57 gene.

10

FIG. 13. Consensus nucleotide sequence of band 105 (SEQ ID NO:5). "N" signifies "any nucleotide". The gene corresponding to band 105 is referred to herein as the 105 gene.

15

FIG. 14. Nucleotide sequence obtained from clone H of band 106 (SEQ ID NO:6). "N" signifies "any nucleotide". The gene corresponding to band 106 is referred to herein as the 106 gene.

20

FIG. 15. Nucleotide sequence of clone G of band 161 (SEQ ID NO:7). The gene corresponding to band 161 is referred to herein as the 161 gene.

25

FIG. 16. Multiple sequence alignment of 161 clone G with amino acid sequences identified in a BLAST search. Asterisks signify positions that are identical; dots indicate conserved positions.

30

FIG. 17. Nucleotide and amino acid sequence of the full length murine 200 gene. Bottom line: murine 200 gene nucleotide sequence (SEQ ID NO:8); top line: murine 200 gene product derived amino acid sequence (SEQ ID NO: 10).

35

FIG. 18. Northern blot analysis of murine 200 gene expression in representative murine TH cell lines (TH2: CDC25, D10.G4, DAX; TH1: AE7.A, Dorris, D1.1). Clones were

either unstimulated (-) or stimulated (+) for 6 hours with plate-bound anti-CD3 antibody. The positions of RNA markers, in kilobases, are shown for reference. The arrow marks the position of 200 gene mRNA.

5

FIG. 19. Northern blot analysis of 54 gene expression within TH1 (D1.1, Dorris, AE7) cell lines and TH2 (D10.G4, DAX, CDC25) cell lines, either stimulated (+) or unstimulated (-) with anti-CD3 antibodies. 15 micrograms of total RNA were loaded per lane. Cells were stimulated between 6 and 7 hours with anti-CD3 antibodies, as described, below, in Section 8.1. The Northern blots were hybridized with a probe made from the entire band 54 nucleotide sequence.

15 FIG. 20. Northern blot analysis of gene 54 time course study. RNA from TH1 cell line AE7 cells was isolated, either unstimulated or stimulated for varying periods of time, as indicated. Second, RNA from two TH2 cell lines (DAX, CDC25) was isolated from either unstimulated cells or from cells 20 which had been stimulated for two hours with anti-CD3 antibodies. 15 micrograms total RNA were loaded per lane. A band 54 DNA probe was used for hybridization.

FIG. 21. Northern blot analysis of 54 gene expression 25 in various tissues. 15 micrograms of total RNA were loaded per lane. A band 54 DNA probe was used for hybridization.

FIG. 22. Nucleotide and amino acid sequence of the full length 54 gene. Bottom line: 54 gene nucleotide sequence 30 (SEQ ID NO:11). Top line: 54 gene derived amino acid sequence (SEQ ID NO:12).

FIG. 23. The 54 gene product bears a high level of homology to the cysteine protease class of proteins. The 54 35 gene product amino acid is depicted with its predicted pre-pro sequence and mature cysteine protease polypeptide sequence identified. The individual boxed amino acid

residues represent residues thought to lie within the cysteine protease active site and the stretch of amino acid residues which are boxed represent a region with homology to a stretch of amino acid residues normally seen within the preproenzyme portion of cysteine protease molecules. The circled amino acid residues within this stretch represent conserved amino acids. The arrow indicates the putative post-translational cleavage site.

10 FIG. 24. Nucleotide and amino acid sequence of the full length human 200 gene. Bottom line: human 200 gene nucleotide sequence (SEQ ID NO: 23); top line: human 200 gene product derived amino acid sequence (SEQ ID NO:24).

15 FIG. 25. Flow cytometry data demonstrates that the 3E10 mAb recognizes and binds to representative clones of the TH2 cell subpopulation (D10.G4; DAX), but not clones of the TH1 subtype (AE7; Dorris). The graphs in this figure present the results of the flow cytometry analyses by depicting the
20 number of cells exhibiting a given level of fluorescence. Staining above background levels represents antigen-specific binding and, therefore, the presence of cell surface 103 gene product. The further to the right the peaks are shifted, the greater the staining intensity, and therefore antibody
25 binding, exhibited by a cell population.

Fig. 26. Analysis of the cytokine profile in mouse BAL. The data presented in this figure reveals high levels of IL-4, IL-5, IL-6, IL-10 and IL-13 in TH2 recipient OVA
30 challenged mice (closed bars). There was no detectable TH2 cytokines in the BAL fluid of mice that received TH2 cells and were not exposed to ovalbumin. Pretreatment with 3E10 mAb resulted in a dramatic reduction in IL-4, IL-5, IL-6 and IL-13, but had no effect on IL-10 levels in the BAL (open
35 bars). OVA challenge of TH1 recipient mice resulted in high levels of IFN- γ in the BAL fluid (closed bars) that was not

inhibited by 3E10 mAb (open bars). Data are shown as the mean \pm sem of 5-6 animals.

- Fig. 27A-27B. Anti-103 gene product mAb inhibits TH2 mediated allergic lung inflammation. FIG. 27A: Analysis of the number of eosinophils in the BAL; FIG. 27B: analysis of the number of lymphocytes in the BAL. The number of OVA-specific TH2 cells in dispersed lung tissue as described (Cohn, L. et al., 1997, J. Exp. Med. 186:1737-1747).
- 10 Lymphocytes were stained with biotinylated clonotypic TCR mAb KJ126 (Cohn, L. et al., 1997 J. Exp. Med. 186:1737-1747) followed by streptavidin-FITC and CD4-PE (Pharmingen, San Diego).
- 15 FIG. 28. Inhibition of airway hyperresponsiveness by anti-103 gene product mAb. OVA exposure in TH2 recipient mice resulted in airway hyperresponsiveness (closed squares) compared to mice exposed to PBS (closed circles). Pretreatment with 103 gene product mAb inhibited OVA induced
- 20 BHR by 80% (open diamonds). The results are shown as the mean \pm sem of $n = 5-6$ and is representative of 2 separate experiments.

- Fig. 29A-29B. Administration of 3E10 mAb or the 103/Ig fusion results in significant decrease in hallmark symptoms of asthma. FIG. 29A: Animals were treated with the anti-103 3E10 antibody (listed in the figure as "3E10 mAb"). As a negative control, a set of animals was treated with a non-specific rat Ig antibody preparation. FIG. 29B: Animals
- 30 were treated with 103/Ig fusion protein (listed in the figure as "Ig Fus. Prot.") as a negative control, a control set of animals were treated with a non-specific human IgG antibody preparation.

- 35 Fig. 30. Crosslinking of 103 gene product augments IL-4 and IL-5 cytokine secretion. TH2 effector cells were activated with plate-bound CD3 (3 μ g/ml, 2C11) and CD28

(37.51, 4 μ g/ml, Pharmingen San Diego) and 3E10 (20 μ g/ml) for 48 hrs. IL-4 and IL-5 levels were measured in the supernatant by ELISA. 3E10 mAb stimulation alone failed to induce TH2 cell activation but augmented both anti-CD3 and 5 anti-CD3 + CD28 induced cytokine production. Soluble 3E10 failed to have any effect on CD3/CD28 mediated cytokine production. These data suggest that activation of 103 gene product provides a stimulatory signal to TH2 cells. There was no effect of the mAb on TH2 cell proliferation as 10 revealed by 3 H-thymidine incorporation. 3E10 mAb did not modify IFN- γ secretion from TH1 effector cells stimulated under the same conditions.

Fig. 31. Renal histology at 72 hours reperfusion; Fig. 15 31A shows a section of untreated mouse kidney tissue; Fig. 31B shows a section of mouse kidney tissue treated with 200 gene antibody 24 hours prior to, and at 24 hour intervals after the induction of ischemic kidney injury.

20 Fig. 32. Histological scoring of gene 200 blockage in treated (+a200) and untreated (+RtIg) mouse kidney tissue during renal ischemia/reperfusion injury (RI), and in non ischemic controls (S).

25 5. DETAILED DESCRIPTION OF THE INVENTION

Methods and compositions for the treatment and diagnosis of immune disorders, especially TH cell subpopulation-related disorders, including, but not limited to, atopic conditions, such as asthma and allergy, including allergic rhinitis, 30 psoriasis, the effects of pathogen infection, chronic inflammatory diseases, organ-specific autoimmunity, graft rejection and graft versus host disease, are described. The methods and compositions described herein can also be used to treat ischemic disorders and injuries, including but not 35 limited to, ischemic renal disease and injury, myocardial ischemia such as angina pectoris, as well as ischemic injury to other tissues, including the brain (as in a stroke),

spleen, intestine, lung, and testes. Further, the methods and compositions described herein can also be used to regulate ischemic injury to other types of tissue, such as tumor tissue including, but not limited to tumors of the ovary and uterus. The invention is based, in part, on the evaluation of the expression and role of all genes that are differentially expressed within and/or among TH cell subpopulations in paradigms that are physiologically relevant to TH-mediated immune response and/or TH-subpopulation related disorders. This permits the definition of disease pathways that are useful both diagnostically and therapeutically. The invention is also based in part on the discovery that the genes and gene products of the invention are also involved in processes related to tissue repair and remodeling after injury, particularly after ischemic injury. Thus, the genes and gene products of the invention can also be used to successfully treat such injuries and related disorders.

Genes, termed "target genes" and/or "fingerprint genes", which are differentially expressed within and among TH cells and TH cell subpopulations in normal and/or disease states, and/or during the differentiation into such mature subpopulations are described in Section 5.4. Additionally, genes, termed "pathway genes", whose gene products exhibit an ability to interact with gene products involved in TH cell subpopulation-related disorders and/or with gene products which are involved in the differentiation and effector function of the subpopulations are described in Section 5.4. Pathway genes can additionally have fingerprint and/or target gene characteristics. Methods for the identification of such fingerprint, target, and pathway genes are also described in Sections 5.1 and 5.2.

Further, the gene products of such fingerprint, target, and pathway genes are described in Section 5.5, antibodies to such gene products are described in Section 5.6, as are cell- and animal-based models of TH cell subpopulation

differentiation and TH cell subpopulation-related disorders to which such gene products can contribute in Section 5.7.

Methods for prognostic and diagnostic evaluation of various TH cell subpopulation-related disorders, for the
5 identification of subjects exhibiting a predisposition to such disorders, and for monitoring the efficacy of compounds used in clinical trials are described in Section 5.12.

Methods for the identification of compounds which modulate the expression of genes or the activity of gene
10 products involved in (a) TH cell subpopulation-related disorders, (b) the differentiation and effector function of TH cell subpopulations, and (c) processes related to tissue repair and remodeling after ischemic injury are described in Section 5.8. Methods for the treatment of immune disorders
15 and ischemic disorders and injuries are described in Section 5.10.

5.1. IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES

Described herein are methods for the identification of
20 differentially expressed genes which are involved in immune disorders, e.g., TH cell subpopulation-related disorders, and/or which are involved in the differentiation, maintenance and effector function of the subpopulations. There exist a number of levels at which the differential expression of such
25 genes can be exhibited. For example, differential expression can occur in undifferentiated TH cells versus differentiated or differentiating TH cells (although not necessarily within one TH cell subpopulation versus another), in naive TH cells versus memory TH cells, within one TH cell subpopulation
30 versus another (e.g., TH1 versus TH2 subpopulations), in mature, stimulated cells versus mature, unstimulated cells of a given TH cell subpopulation or in TH cell subpopulation-related disorder states relative to their expression in normal, or non-TH cell subpopulation-related disorder states.
35 Such differentially expressed genes can represent target and/or fingerprint genes.

Methods for the identification of such differentially expressed genes are described, below, in Section 5.1.1. Methods for the further characterization of such differentially expressed genes, and for their categorization as target and/or fingerprint genes, are presented, below, in Section 5.3.

"Differential expression" as used herein refers to both quantitative as well as qualitative differences in the genes' temporal and/or cell type expression patterns. Thus, a differentially expressed gene can qualitatively have its expression activated or completely inactivated in, for example, normal versus TH cell subpopulation-related disorder states, in one TH cell subpopulation versus another (e.g., TH1 versus TH2), in antigen stimulated versus unstimulated sets of TH cells, or in undifferentiated versus differentiated or differentiating TH cells. Such a qualitatively regulated gene will exhibit an expression pattern within a state or cell type which is detectable by standard techniques in one such state or cell type, but is not detectable in both.

Alternatively, a differentially expressed gene can exhibit an expression level which differs, i.e., is quantitatively increased or decreased, in normal versus TH cell subpopulation-related disorder states, in antigen stimulated versus unstimulated sets of TH cells, in one TH cell subpopulation versus another, or in undifferentiated versus differentiated or differentiating TH cells. Because differentiation is a multistage event, genes which are differentially expressed can also be identified at any such intermediate differentiative stage.

The degree to which expression differs need only be large enough to be visualized via standard characterization techniques, such as, for example, the differential display technique described below. Other such standard characterization techniques by which expression differences can be visualized include, but are not limited to,

quantitative RT (reverse transcriptase) PCR and Northern analyses and RNase protection techniques.

Differentially expressed genes can be further described as target genes and/or fingerprint genes. "Fingerprint gene," as used herein, refers to a differentially expressed gene whose expression pattern can be utilized as part of a prognostic or diagnostic TH cell subpopulation-related disorder evaluation, or which, alternatively, can be used in methods for identifying compounds useful for the treatment of TH cell subpopulation-related disorders. A fingerprint gene can also have the characteristics of a target gene or a pathway gene (see below, in Section 5.2).

"Fingerprint pattern," as used herein, refers to the pattern generated when the expression pattern of a series (which can range from two up to all the fingerprint genes which exist for a given state) of fingerprint genes is determined. A fingerprint pattern can also be used in methods for identifying compounds useful in the treatment of immune disorders, e.g., by evaluating the effect of the compound on the fingerprint pattern normally displayed in connection with the disease.

"Target gene", as used herein, refers to a differentially expressed gene involved in TH cell subpopulation-related disorders and/or in differentiation, maintenance and/or effector function of the subpopulations in a manner by which modulation of the level of target gene expression or of target gene product activity can act to ameliorate symptoms of TH cell subpopulation-related disorders. For example, such modulation can result either the depletion or stimulation of one or more TH cell subpopulation, which, in turn, brings about the amelioration of immune disorder, e.g., TH cell subpopulation disorder, symptoms.

"Stimulation", as used herein, can refer to an effective increase in the number of cells belonging to a T cell population, such as a TH cell subpopulation, via, for example, the proliferation of such TH cell subpopulation

cells. The term can also refer to an increase in the activity of cells belonging to a TH cell subpopulation, as would be evidenced, for example, by a per cell increase in the expression of the TH cell subpopulation-specific cytokine pattern.

"Depletion", as used herein, can refer to an effective reduction in the number of cells belonging to a T cell population, such as a TH cell subpopulation, via, for example, a reduction in the proliferation of such TH cell subpopulation cells. The term can also refer to a decrease in the activity of cells belonging to a TH cell subpopulation, as would be evidenced, for example, by a per cell decrease in the expression of the TH cell subpopulation-specific cytokine pattern.

TH cell subpopulation-related disorders include, for example, atopic conditions, such as asthma and allergy, including allergic rhinitis, the effects of pathogen, including viral, infection, chronic inflammatory diseases, psoriasis, glomerular nephritis, organ-specific autoimmunity, graft rejection and graft versus host disease. A target gene can also have the characteristics of a fingerprint gene and/or a pathway gene (as described, below, in Section 5.2).

5.1.1. METHODS FOR THE IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES

A variety of methods can be utilized for the identification of genes which are involved in immune disorder states, e.g., TH cell subpopulation-related disorder states, and/or which are involved in differentiation, maintenance and/or effector function of the subpopulations. Described in Section 5.1.1.1 are experimental paradigms which can be utilized for the generation of subjects and samples which can be used for the identification of such genes. Material generated in paradigm categories can be characterized for the presence of differentially expressed gene sequences as discussed, below, in Section 5.1.1.2.

5.1.1.1. PARADIGMS FOR THE IDENTIFICATION
OF DIFFERENTIALLY EXPRESSED GENES

Paradigms which represent models of normal and abnormal immune responses are described herein. These paradigms can be utilized for the identification of genes which are differentially expressed within and among TH cell subpopulations, including but not limited to TH1 and TH2 subpopulations. Such genes can be involved in, for example, TH cell subpopulation differentiation, maintenance, and/or effector function, and in TH cell subpopulation-related disorders. For example, TH cells can be induced to differentiate into either TH1 or TH2 states, can be stimulated with, for example, a foreign antigen, and can be collected at various points during the procedure for analysis of differential gene expression.

In one embodiment of such a paradigm, referred to herein as the "transgenic T cell paradigm", transgenic animals, preferably mice, are utilized which have been engineered to express a particular T cell receptor, such that the predominant T cell population of the immune system of such a transgenic animal recognizes only one antigen. Such a system is preferred in that it provides a source for a large population of identical T cells whose naivete can be assured, and whose response to the single antigen it recognizes is also assured. T helper cells isolated from such a transgenic animal are induced, in vitro, to differentiate into TH cell subpopulations such as TH1, TH2, or TH0 cell subpopulations. In a specific embodiment, one T helper cell group (the TH1 group) is exposed to IL-12, a cytokine known to induce differentiation into the TH1 state, a second T helper cell group (the TH2 group) is exposed to IL-4, a cytokine known to induce differentiation into the TH2 state, and a third group is allowed, by a lack of cytokine-mediated induction, to enter a TH-undirected state.

A second paradigm, referred to herein as a "T cell line paradigm", can be utilized which uses mature TH cell clones, such as TH1 and TH2 and TH1-like and TH2-like cell lines,

preferably human cell lines. Such TH cell lines can include, but are not limited to the following well known murine cell lines: Doris, AE7, D10.G4, DAX, D1.1 and CDC25. Such T cell lines can be derived from normal individuals as well as
5 individuals exhibiting TH cell subpopulation-related disorders, such as, for example, chronic inflammatory diseases and disorders, such as Crohn's disease, reactive arthritis, including Lyme disease, insulin-dependent diabetes, organ-specific autoimmunity, including multiple
10 sclerosis, Hashimoto's thyroiditis and Grave's disease, contact dermatitis, psoriasis, graft rejection, graft versus host disease, sarcoidosis, atopic conditions, such as asthma and allergy, including allergic rhinitis, gastrointestinal allergies, including food allergies, eosinophilia,
15 conjunctivitis, glomerular nephritis, certain pathogen susceptibilities such as helminthic (e.g., leishmaniasis) and certain viral infections, including HIV, and bacterial infections, including tuberculosis and lepromatous leprosy.

The TH cell clones can be stimulated in a variety of
20 ways. Such stimulation methods include, but are not limited to, pharmacological methods, such as exposure to phorbol esters, calcium ionophores, or lectins (e.g., Concanavalin A), by treatment with antibodies directed against T-cell receptor epitopes (e.g., anti-CD3 antibodies) or exposure, in
25 the presence of an appropriate antigen presenting cell (APC), to an antigen that the particular TH cells are known to recognize. Following such primary stimulation, the cells can be maintained in culture without stimulation and, for example, in the presence of IL-2, utilizing standard
30 techniques well known to those of skill in the art. The cells can then be exposed to one or more additional cycles of stimulation and maintenance.

A third paradigm, referred to herein as an "in vivo paradigm", can also be utilized to discover differentially
35 expressed gene sequences. In vivo stimulation of animal models forms the basis for this paradigm. The in vivo nature of the stimulation can prove to be especially predictive of

the analogous responses in living patients. Stimulation can be accomplished via a variety of methods. For example, animals, such as transgenic animals described earlier in this Section, can be injected with appropriate antigen and

5 appropriate cytokine to drive the desired TH cell differentiation. Draining lymph nodes can then be harvested at various time points after stimulation. Lymph nodes from, for example, TH1-directed animals can be compared to those of TH2-directed animals.

10 A wide range of animal models, representing both models of normal immune differentiation and function as well as those representing immune disorders can be utilized for this in vivo paradigm. For example, any of the animal models, both recombinant and non-recombinant, described, below, in
15 Section 5.7.1, can be used.

Cell samples can be collected during any point of such a procedure. For example, cells can be obtained following any stimulation period and/or any maintenance period.

Additionally, cells can be collected during various points
20 during the TH cell differentiation process. RNA collected from such samples can be compared and analyzed according to, for example, methods described, below, in Section 5.1.1.2. For example, RNA from TH0, TH1 and TH2 groups isolated at a given time point can then be analyzed and compared.

25 Additionally, RNA from stimulated and non-stimulated cells within a given TH cell group can also be compared and analyzed. Further, RNA collected from undifferentiated TH cells can be compared to RNA collected from cells at various stages during the differentiative process which ultimately
30 yields TH cell subpopulations.

5.1.1.2. ANALYSIS OF PARADIGM MATERIAL

In order to identify differentially expressed genes, RNA, either total or mRNA, can be isolated from the TH cells
35 utilized in paradigms such as those described in Section 5.1.1.1. Any RNA isolation technique which does not select against the isolation of mRNA can be utilized for the

purification of such RNA samples. See, for example, Ausubel, F.M. et al., eds., 1987-1993, Current Protocols in Molecular Biology, John Wiley & Sons, Inc. New York, which is incorporated herein by reference in its entirety.

5 Additionally, large numbers of cell samples can readily be processed using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski, P. (1989, U.S. Patent No. 4,843,155), which is incorporated herein by reference in its entirety.

10 Transcripts within the collected RNA samples which represent RNA produced by differentially expressed genes can be identified by utilizing a variety of methods which are well known to those of skill in the art. For example, differential screening (Tedder, T.F. et al., 1988, Proc. Natl. Acad. Sci. USA 85:208-212), subtractive hybridization (Hedrick, S.M. et al., 1984, Nature 308:149-153; Lee, S.W. et al., 1984, Proc. Natl. Acad. Sci. USA 88:2825), and, preferably, differential display (Liang, P. and Pardee, A.B., 1992, Science 257:967-971; U.S. Patent No. 5,262,311, which
15 are incorporated herein by reference in their entirety), can be utilized to identify nucleic acid sequences derived from genes that are differentially expressed.

Differential screening involves the duplicate screening of a cDNA library in which one copy of the library is
25 screened with a total cell cDNA probe corresponding to the mRNA population of one cell type while a duplicate copy of the cDNA library is screened with a total cDNA probe corresponding to the mRNA population of a second cell type. For example, one cDNA probe can correspond to a total cell
30 cDNA probe of a cell type or tissue derived from a control subject, while the second cDNA probe can correspond to a total cell cDNA probe of the same cell type or tissue derived from an experimental subject. Those clones which hybridize to one probe but not to the other potentially represent
35 clones derived from genes differentially expressed in the cell type of interest in control versus experimental subjects.

Subtractive hybridization techniques generally involve the isolation of mRNA taken from two different sources, the hybridization of the mRNA or single-stranded cDNA reverse-transcribed from the isolated mRNA, and the removal of all hybridized, and therefore double-stranded, sequences. The remaining non-hybridized, single-stranded cDNAs, potentially represent clones derived from genes that are differentially expressed among the two mRNA sources. Such single-stranded cDNAs are then used as the starting material for the construction of a library comprising clones derived from differentially expressed genes.

The differential display technique is a procedure, utilizing the well-known polymerase chain reaction (PCR; the experimental embodiment set forth in Mullis, K.B., 1987, U.S. Patent No. 4,683,202), which allows for the identification of sequences derived from genes which are differentially expressed. First, isolated RNA is reverse-transcribed into single-stranded cDNA, utilizing standard techniques which are well known to those of skill in the art. Primers for the reverse transcriptase reaction can include, but are not limited to, oligo dT-containing primers, preferably of the 3' primer type of oligonucleotide described below.

Next, this technique uses pairs of PCR primers, as described below, which allow for the amplification of clones representing a reproducible subset of the RNA transcripts present within any given cell. Utilizing different pairs of primers allows each of the primed mRNA transcripts present in a cell to be amplified. Among such amplified transcripts can be identified those which have been produced from differentially expressed genes.

The 3' oligonucleotide primer of the primer pairs can contain an oligo dT stretch of 10-13, preferably 11, dT nucleotides at its 5' end, which hybridizes to the poly(A) tail of mRNA or to the complement of a cDNA reverse transcribed from an mRNA poly(A) tail. In order to increase the specificity of the 3' primer, the primer can contain one or more, preferably two, additional nucleotides at its 3'

end. Because, statistically, only a subset of the mRNA derived sequences present in the sample of interest will hybridize to such primers, the additional nucleotides allow the primers to amplify only a subset of the mRNA derived
5 sequences present in the sample of interest. This is preferred in that it allows more accurate and complete visualization and characterization of each of the bands representing amplified sequences.

The 5' primer can contain a nucleotide sequence
10 expected, statistically, to have the ability to hybridize to cDNA sequences derived from the cells or tissues of interest. The nucleotide sequence can be an arbitrary one, and the length of the 5' oligonucleotide primer can range from about 9 to about 15 nucleotides, with about 13 nucleotides being
15 preferred.

Arbitrary primer sequences cause the lengths of the amplified partial cDNAs produced to be variable, thus allowing different clones to be separated by using standard denaturing sequencing gel electrophoresis.

20 PCR reaction conditions should be chosen which optimize amplified product yield and specificity, and, additionally, produce amplified products of lengths which can be resolved utilizing standard gel electrophoresis techniques. Such reaction conditions are well known to those of skill in the
25 art, and important reaction parameters include, for example, length and nucleotide sequence of oligonucleotide primers as discussed above, and annealing and elongation step temperatures and reaction times.

The pattern of clones resulting from the reverse
30 transcription and amplification of the mRNA of two different cell types is displayed via sequencing gel electrophoresis and compared. Differentially expressed genes are indicated by differences in the two banding patterns.

Once potentially differentially expressed gene sequences
35 have been identified via bulk techniques such as, for example, those described above, the differential expression of such putatively differentially expressed genes should be

corroborated. Corroboration can be accomplished via, for example, such well known techniques as Northern analysis, quantitative RT/PCR, or RNase protection.

Upon corroboration, the differentially expressed genes
5 can be further characterized, and can be identified as target and/or fingerprint genes, as discussed, below, in Section 5.3.

The amplified sequences of differentially expressed genes obtained through, for example, differential display can
10 be used to isolate full length clones of the corresponding gene. The full length coding portion of the gene can readily be isolated, without undue experimentation, by molecular biological techniques well known in the art. For example, the isolated differentially expressed amplified fragment can
15 be labeled and used to screen a cDNA library. Alternatively, the labeled fragment can be used to screen a genomic library.

PCR technology can also be utilized to isolate full length cDNA sequences. As described, above, in this Section, the isolated, amplified gene fragments obtained through
20 differential display have 5' terminal ends at some random point within the gene and usually have 3' terminal ends at a position corresponding to the 3' end of the transcribed portion of the gene. Once nucleotide sequence information from an amplified fragment is obtained, the remainder of the
25 gene (i.e., the 5' end of the gene, when utilizing differential display) can be obtained using, for example, RT-PCR.

In one embodiment of such a procedure for the identification and cloning of full length gene sequences, RNA
30 can be isolated, following standard procedures, from an appropriate tissue or cellular source. A reverse transcription reaction can then be performed on the RNA using an oligonucleotide primer complimentary to the mRNA that corresponds to the amplified fragment, for the priming of
35 first strand synthesis. Because the primer is anti-parallel to the mRNA, extension will proceed toward the 5' end of the mRNA. The resulting RNA/DNA hybrid can then be "tailed" with

guanines using a standard terminal transferase reaction, the hybrid can be digested with RNAase H, and second strand synthesis can then be primed with a poly-C primer. Using the two primers, the 5' portion of the gene is amplified using 5 PCR. Sequences obtained can then be isolated and recombined with previously isolated sequences to generate a full-length cDNA of the differentially expressed genes of the invention. For a review of cloning strategies and recombinant DNA techniques, see e.g., Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, (Volumes 1-3) Cold Spring Harbor Press, N.Y.; and Ausubel et al., 1989, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y.

15 5.2. METHODS FOR THE IDENTIFICATION OF PATHWAY GENES

Methods are described herein for the identification of pathway genes. "Pathway gene", as used herein, refers to a gene whose gene product exhibits the ability to interact with gene products involved in TH cell subpopulation-related disorders and/or to interact with gene products which are 20 involved in differentiation, maintenance and/or effector function of TH cell subpopulations. A pathway gene can be differentially expressed and, therefore, can have the characteristics of a target and/or fingerprint gene, as 25 described, above, in Section 5.1.

Any method suitable for detecting protein-protein interactions can be employed for identifying pathway gene products by identifying interactions between gene products and gene products known to be involved in TH cell subpopulation-related disorders and/or involved in 30 differentiation, maintenance, and/or effector function of the subpopulations. Such known gene products can be cellular or extracellular proteins. Those gene products which interact with such known gene products represent pathway gene products 35 and the genes which encode them represent pathway genes.

Among the traditional methods which can be employed are co-immunoprecipitation, crosslinking and co-purification

through gradients or chromatographic columns. Utilizing procedures such as these allows for the identification of pathway gene products. Once identified, a pathway gene product can be used, in conjunction with standard techniques, to identify its corresponding pathway gene. For example, at least a portion of the amino acid sequence of the pathway gene product can be ascertained using techniques well known to those of skill in the art, such as via the Edman degradation technique (see, e.g., Creighton, 1983, "Proteins: Structures and Molecular Principles", W.H. Freeman & Co., N.Y., pp.34-49). The amino acid sequence obtained can be used as a guide for the generation of oligonucleotide mixtures that can be used to screen for pathway gene sequences. Screening can be accomplished, for example, by standard hybridization or PCR techniques. Techniques for the generation of oligonucleotide mixtures and for screening are well-known. (See, e.g., Ausubel, supra., and PCR Protocols: A Guide to Methods and Applications, 1990, Innis, M. et al., eds. Academic Press, Inc., New York).

Additionally, methods can be employed which result in the simultaneous identification of pathway genes which encode proteins interacting with a protein involved in TH cell subpopulation-related disorder states and/or differentiation, maintenance, and/or effector function of the subpopulations. These methods include, for example, probing expression libraries with labeled protein known or suggested to be involved in the disorders and/or the differentiation, maintenance, and/or effector function of the subpopulations, using this protein in a manner similar to the well known technique of antibody probing of λ gt11 libraries.

One method which detects protein interactions in vivo, the two-hybrid system, is described in detail for illustration purposes only and not by way of limitation. One version of this system has been described (Chien et al., 1991, Proc. Natl. Acad. Sci. USA, 88:9578-9582) and is commercially available from Clontech (Palo Alto, CA).

Briefly, utilizing such a system, plasmids are constructed that encode two hybrid proteins: one consists of the DNA-binding domain of a transcription activator protein fused to a known protein, in this case, a protein known to be
5 involved in TH cell subpopulation differentiation or effector function, or in TH cell subpopulation-related disorders, and the other consists of the activator protein's activation domain fused to an unknown protein that is encoded by a cDNA which has been recombined into this plasmid as part of a cDNA
10 library. The plasmids are transformed into a strain of the yeast Saccharomyces cerevisiae that contains a reporter gene (e.g., lacZ) whose regulatory region contains the transcription activator's binding sites. Either hybrid protein alone cannot activate transcription of the reporter
15 gene, the DNA-binding domain hybrid cannot because it does not provide activation function, and the activation domain hybrid cannot because it cannot localize to the activator's binding sites. Interaction of the two hybrid proteins reconstitutes the functional activator protein and results in
20 expression of the reporter gene, which is detected by an assay for the reporter gene product.

The two-hybrid system or related methodology can be used to screen activation domain libraries for proteins that interact with a known "bait" gene product. By way of
25 example, and not by way of limitation, gene products known to be involved in TH cell subpopulation-related disorders and/or differentiation, maintenance, and/or effector function of the subpopulations can be used as the bait gene products. Total genomic or cDNA sequences are fused to the DNA encoding an
30 activation domain. This library and a plasmid encoding a hybrid of the bait gene product fused to the DNA-binding domain are cotransformed into a yeast reporter strain, and the resulting transformants are screened for those that express the reporter gene. For example, and not by way of
35 limitation, the bait gene can be cloned into a vector such that it is translationally fused to the DNA encoding the DNA-binding domain of the GAL4 protein. These colonies are

purified and the library plasmids responsible for reporter gene expression are isolated. DNA sequencing is then used to identify the proteins encoded by the library plasmids.

A cDNA library of the cell line from which proteins that interact with bait gene product are to be detected can be made using methods routinely practiced in the art. According to the particular system described herein, for example, the cDNA fragments can be inserted into a vector such that they are translationally fused to the activation domain of GAL4.

10 This library can be co-transformed along with the bait gene-GAL4 fusion plasmid into a yeast strain which contains a lacZ gene driven by a promoter which contains GAL4 activation sequence. A cDNA encoded protein, fused to GAL4 activation domain, that interacts with bait gene product will

15 reconstitute an active GAL4 protein and thereby drive expression of the lacZ gene. Colonies which express lacZ can be detected by their blue color in the presence of X-gal. The cDNA can then be purified from these strains, and used to produce and isolate the bait gene-interacting protein using

20 techniques routinely practiced in the art.

Once a pathway gene has been identified and isolated, it can be further characterized as, for example, discussed below, in Section 5.3.

25 5.3. CHARACTERIZATION OF DIFFERENTIALLY EXPRESSED AND PATHWAY GENES

Differentially expressed genes, such as those identified via the methods discussed, above, in Section 5.1, and pathway genes, such as those identified via the methods discussed,

30 above, in Section 5.2, above, as well as genes identified by alternative means, can be further characterized by utilizing, for example, methods such as those discussed herein. Such genes will be referred to herein as "identified genes".

Analyses such as those described herein yield

35 information regarding the biological function of the identified genes. An assessment of the biological function of the differentially expressed genes, in addition, will

allow for their designation as target and/or fingerprint genes.

Specifically, any of the differentially expressed genes whose further characterization indicates that a modulation of the gene's expression or a modulation of the gene product's activity can ameliorate any of the TH cell subpopulation-related disorders of interest will be designated "target genes", as defined, above, in Section 5.1. Such target genes and target gene products, along with those discussed below, will constitute the focus of the compound discovery strategies discussed, below, in Section 5.8. Further, such target genes, target gene products and/or modulating compounds can be used as part of the TH cell subpopulation-disorder treatment methods described, below, in Section 5.9. Such methods can include, for example, methods whereby the TH cell subpopulation of interest is selectively depleted or repressed, or, alternatively, stimulated or augmented.

Any of the differentially expressed genes whose further characterization indicates that such modulations can not positively affect TH cell subpopulation-related disorders of interest, but whose expression pattern contributes to a gene expression "fingerprint" pattern correlative of, for example, a TH1/TH2-related disorder state, will be designated a "fingerprint gene". "Fingerprint patterns" will be more fully discussed, below, in Section 5.12.1. It should be noted that each of the target genes can also function as fingerprint genes, as well as can all or a portion of the pathway genes.

It should further be noted that the pathway genes can also be characterized according to techniques such as those described herein. Those pathway genes which yield information indicating that modulation of the gene's expression or a modulation of the gene product's activity can ameliorate any a TH cell subpopulation-related disorder will also be designated "target genes". Such target genes and target gene products, along with those discussed above, will constitute the focus of the compound discovery strategies

discussed, below, in Section 5.8 and can be used as part of the treatment methods described in Section 5.9, below.

In instances wherein a pathway gene's characterization indicates that modulation of gene expression or gene product activity can not positively affect TH cell subpopulation-related disorders of interest, but whose expression is differentially expressed and contributes to a gene expression fingerprint pattern correlative of, for example, a TH1/TH2-related disorder state, such pathway genes can additionally be designated as fingerprint genes.

A variety of techniques can be utilized to further characterize the identified genes. First, the nucleotide sequence of the identified genes, which can be obtained by utilizing standard techniques well known to those of skill in the art, can, for example, be used to reveal homologies to one or more known sequence motifs which can yield information regarding the biological function of the identified gene product.

Second, an analysis of the tissue and/or cell type distribution of the mRNA produced by the identified genes can be conducted, utilizing standard techniques well known to those of skill in the art. Such techniques can include, for example, Northern, RNase protection, and RT-PCR analyses. Such analyses provide information as to, for example, whether the identified genes are expressed in cell types expected to contribute to the specific TH cell subpopulation-related disorders of interest. Such analyses can also provide quantitative information regarding steady state mRNA regulation, yielding data concerning which of the identified genes exhibits a high level of regulation in cell types which can be expected to contribute to the TH cell subpopulation-related disorders of interest. Additionally, standard in situ hybridization techniques can be utilized to provide information regarding which cells within a given tissue or population of cells express the identified gene. Such an analysis can provide information regarding the biological function of an identified gene relative to a given TH cell

subpopulation-related disorder in instances wherein only a subset of the cells within a tissue or a population of cells is thought to be relevant to the disorder.

- Third, the sequences of the identified genes can be used, utilizing standard techniques, to place the genes onto genetic maps, e.g., mouse (Copeland, N.G. and Jenkins, N.A., 1991, Trends in Genetics 7:113-118) and human genetic maps (Cohen, D., et al., 1993, Nature 366:698-701). Such mapping information can yield information regarding the genes' importance to human disease by, for example, identifying genes which map within genetic regions to which known genetic TH cell subpopulation-related disorders map. Such regions include, for example, the mouse Scl-1 locus, which is suspected to be involved in Leishmaniasis, or the human 5q31.1 chromosomal region which contains one or more loci thought to regulate IgE production in a nonantigen-specific fashion, and can, therefore, be involved in allergy, a TH2-like-related disorder (Marsh, D. et al., 1994, Science 264:1152-1156).
- Fourth, the biological function of the identified genes can be more directly assessed by utilizing relevant in vivo and in vitro systems. In vivo systems can include, but are not limited to, animal systems which naturally exhibit the symptoms of immune disorders, or ones which have been engineered to exhibit such symptoms. Further, such systems can include systems for the further characterization of the cell type differentiation and effector function, and can include, but are not limited to transgenic animal systems such as those described, above, in Section 5.1.1.1, and Section 5.7.1, below. In vitro systems can include, but are not limited to, cell-based systems comprising, for example, TH1 or TH2 cell types. The TH subpopulation cells can be wild type cells, or can be non-wild type cells containing modifications known or suspected of contributing to the TH cell subpopulation-related disorder of interest. Such systems are discussed in detail, below, in Section 5.7.2.

In further characterizing the biological function of the identified genes, the expression of these genes can be modulated within the in vivo and/or in vitro systems, i.e., either overexpressed or underexpressed in, for example, transgenic animals and/or cell lines, and its subsequent effect on the system can then be assayed. Alternatively, the activity of the product of the identified gene can be modulated by either increasing or decreasing the level of activity in the in vivo and/or in vitro system of interest, and its subsequent effect then assayed.

The information obtained through such characterizations can suggest relevant methods for the treatment or control of immune disorders, such as TH cell subpopulation-related disorders, involving the gene of interest. For example, relevant treatment can include not only a modulation of gene expression and/or gene product activity, but can also include a selective depletion or stimulation of the TH cell subpopulation of interest. Characterization procedures such as those described herein can indicate where such modulation should be positive or negative. As used herein, "positive modulation" refers to an increase in gene expression or activity of the gene or gene product of interest, or to a stimulation of a TH cell subpopulation, relative to that observed in the absence of the modulatory treatment. "Negative modulation", as used herein, refers to a decrease in gene expression or activity, or a depletion of a TH cell subpopulation, relative to that observed in the absence of the modulatory treatment. "Stimulation" and "depletion" are as defined, above, in Section 3. Methods of treatment are discussed, below, in Section 5.9.

5.4. DIFFERENTIALLY EXPRESSED AND PATHWAY GENES

Differentially expressed genes such as those identified in Section 5.1.1, above, and pathway genes, such as those identified in Section 5.2, above, are described herein.

The differentially expressed and pathway genes of the invention are listed below, in Table 1. Differentially

expressed gene sequences are shown in FIGS. 2, 4A, 9 and 12-15, 17, 22 and 24. The nucleotide sequences identified via differential display analysis are referred to herein as band 10, 54, 57, 102, 103, 105, 106, 161 and 200. The genes corresponding to these sequences are referred to herein as the 10, 54, 57, 102, 103, 106, 161 and 200 genes, respectively. Table 1 lists differentially expressed genes identified through, for example, the paradigms discussed, above, in Section 5.1.1.1, and below, in the Examples presented in Sections 6-8.

Table 1 summarizes information regarding the further characterization of such genes. Table 2 lists E. coli clones, deposited with the Agricultural Research Service Culture Collection (NRRL) or the American Type Culture Collection (ATCC), which contain sequences found within the genes of Table 1.

In Table 1, the column headed "Diff. Exp." details the differential expression characteristic by which the sequence has been identified. Under this column, "TH Inducible", refers to those cases where differential expression arises upon exposure of the cell type of interest to an agent capable of bringing about TH cell stimulation or activation. These sequences, therefore, are differentially expressed in undifferentiated, partially or fully differentiated TH cells, and the genes corresponding to these sequences are expressed in both TH1 and TH2 cell subpopulations.

"TH1", under this column, refers to a sequence corresponding to a gene expressed preferentially in mature, fully differentiated TH1 cells relative to TH2 cells. "TH2", under this column, refers to a sequence corresponding to a gene preferentially expressed in mature, fully differentiated TH2 cell subpopulations relative to TH1 cell subpopulations. Preferential expression can be qualitative or quantitative, as described, above, in Section 5.1.

Tissue expression patterns are also summarized in Table 1. The column headed "Tissue/Cell Dist." lists tissues and/or cell types in which expression of the gene has been

tested and whether expression of the gene within a given tissue or cell type has been observed. Specifically, "+" indicates detectable mRNA from the gene of interest, while "-" refers to no detectable mRNA from the gene of interest.

5 Unless otherwise noted, "+" and "-" refer to all samples of a given tissue or cell type tested. "Detectable", as used herein, is as described, above, in Section 5.1.

Additionally, the physical locus to which the gene maps on the human and/or mouse chromosome map is indicated in the
10 column headed "Locus". Further, in instances wherein the genes correspond to genes known to be found in nucleic acid databases, references (*i.e.*, citations and/or gene names) to such known genes are listed in the column headed "Ref.".

The genes listed in Table 1 can be obtained using
15 cloning methods well known to those of skill in the art, and include but are not limited to the use of appropriate probes to detect the genes within an appropriate cDNA or gDNA (genomic DNA) library. (See, for example, Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring
20 Harbor Laboratories, which is incorporated herein by reference in its entirety.) Probes for the sequences reported herein can be obtained directly from the isolated clones deposited with the NRRL, as indicated in Table 2, below. Alternatively, oligonucleotide probes for the genes
25 can be synthesized based on the DNA sequences disclosed herein in FIGS. 2, 4A, 9, 12-15, 17, 22 and 24. With respect to the previously reported genes, synthetic oligonucleotides can be synthesized or produced based on the sequences provided for the previously known genes described in the
30 following references: granzyme A, Hanukah factor: Masson, D. et al., 1986, FEBS Lett. 208:84-88; Masson, D. et al., 1986, EMBO J. 5:1595-1600; Gershensfeld, H.K. and Weissman, I.L., 1986, Science 232:854-858; ST-2, T1, Fit-1: Klemenz, R. et al., 1989, Proc. Natl. Acad. Sci. USA 86:5708-5712;
35 Tominaga, S., 1989, FEBS Lett. 258:301-301; Werenskiold, A.K. et al., 1989, Mol. Cell. Biol. 9:5207-5214; Tominaga, S. et al., 1992, Biochem. Biophys. Acta. 1171:215-218; Werenskiold,

A.K., 1992, Eur. J. Biochem. 204:1041-1047; Yanagisawa, K. et al., 1993, FEBS Lett. 318:83-87; Bergers, G. et al., 1994, EMBO J. 13:1176-1188; and Kumar, S., 1997, Biochem. Biophys. Res. Comm. 235:474-478.

- 5 The probes can be used to screen cDNA libraries prepared from an appropriate cell or cell line in which the gene is transcribed. Appropriate cell lines can include, for example, Dorris, AE7, D10.G4, DAX, D1.1 and CDC25 cell lines. In addition, purified primary naive T cells derived from
- 10 either transgenic or non-transgenic strains can be used. Alternatively, the genes described herein can be cloned from a cDNA library constructed from, for example, NIH 3T3 cell lines stably transfected with the Ha-ras(EJ) gene, 5C10 cells, and peripheral blood lymphocytes.

15

20

25

30

35

TABLE 1
DIFFERENTIALLY EXPRESSED AND PATHWAY GENES

Gene	Diff. Exp.	Tissue/ Cell Dist.	Locus	Ref
102	TH2	TH2 Specific		ref1
103	TH2	(+) TH2 (-) Lymph Node; Spleen; Thymus; Brain; Lung; Bone Marrow; Heart; Spleen.		ref2
10	TH Inducible	(+) Spleen; TH1; TH2. (-) Liver; Brain; Thymus; Bone Marrow; Heart; Lymph Node.	See FIG. 11	
57	TH Inducible	(+) TH1; TH2; Spleen		
105	TH1	(+) TH1; Spleen		
106	TH1	(+) TH1; Thymus; Spleen		
161	Subset Specific ¹	(+) Spleen (-) Thymus		
200	TH1	(+) TH1		
54	TH1	(+) TH1; spleen; testis; uterus (-) brain; heart; kidney; liver; muscle		

¹ Masson, D. et al., 1986, FEBS Lett. 208:84-88;
Masson, D. et al., 1986, EMBO J. 5:1595-1600; Gershenfeld,
H.K. and Weissman, I.L., 1986, Science 232:854-858.

² Klemenz, R. et al., 1989, Proc. Natl. Acad. Sci. USA 86:5708-5712; Tominaga, S., 1989, FEBS Lett. 258:301-301; Werenskiold, A.K. et al., 1989, Mol. Cell. Biol. 9:5207-5214; Tominaga, S. et al., 1992, Biochem. Biophys. Acta. 1171:215-218; Werenskiold, A.K., 1992, Eur. J. Biochem. 204:10-1047; Yanagisawa, K. et al., 1993, FEBS Lett. 318:83-87; Bergers, G. et al., 1994, EMBO J. 13:1176-1188 and Kumar, S., 1997, Biochem Biophys Res Commun 235:474-478.

³ Band 161 expression has been observed in either TH1 or TH2 cell subpopulations, but has not been found, simultaneously, in both TH1 and TH2 cell subpopulations.

10

Table 2, below, lists isolated E. coli clones which contain sequences within the novel genes listed in Table 1.

TABLE 2

15

20

25

30

GENE	CLONE
10	10-C
10	10-X
57	57-E
105	105-A
106	106-H
161	161-G
200 (murine)	200-O
200 (murine)	DH10B(Zip) TM containing 200-P
200 (murine)	200-AF
200 (human)	feht 200-C
54	54-C
200 (human)	feht 200-C

As used herein, "differentially expressed gene" (i.e. target and fingerprint gene) or "pathway gene" refers to (a) a gene containing: at least one of the DNA sequences and/or fragments thereof that are disclosed herein (as shown in FIGS. 2, 4A, 9, 12-15, 17, 22 and 24), or contained in the clones listed in Table 2, as deposited with the NRRL or ATCC; (b) any DNA sequence or fragment thereof that encodes the

amino acid sequence encoded by: the DNA sequences disclosed herein (as shown in FIGS. 2, 4A, 9, 12-15, 17, 22 and 24), contained in the clones, listed in Table 2, as deposited with the NRRL or ATCC contained within the coding region of the gene to which the DNA sequences disclosed herein (as shown in FIGS. 2, 4A, 9, 12-15, 17, 22 and 24) belong or contained in the clones listed in Table 2, as deposited with the NRRL or ATCC, belong; (c) any DNA sequence that hybridizes to the complement of: the coding sequences disclosed herein (as shown in FIGS. 2, 4A, 9, 12-15, 17, 22 and 24), contained in clones listed in Table 2, as deposited with the NRRL or ATCC, or contained within the coding region of the gene to which the DNA sequences disclosed herein (as shown in FIGS. 2, 4A, 9, 12-15, 17, 22 and 24) belong or contained in the clones listed in Table 2, as deposited with the NRRL or ATCC, under stringent conditions, e.g., hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45°C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65°C, or under highly stringent conditions, e.g., hybridization to filter-bound nucleic acid in 6xSSC at about 45°C followed by one or more washes in 0.1xSSC/0.2% SDS at about 68°C, or under other hybridization conditions which are apparent to those of skill in the art (see, for example, Ausubel, F.M. et al., eds., 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York, at pp. 6.3.1-6.3.6 and 2.10.3).

Preferably, the nucleic acid molecules that hybridize to the complements of the DNA sequences disclosed herein encode gene products, e.g., gene products that are functionally equivalent to a gene product encoded by a gene of (a), above.

"Functionally equivalent", as utilized herein, refers to a protein capable of exhibiting a substantially similar in vivo activity as the endogenous differentially expressed or pathway gene products encoded by the differentially expressed or pathway gene sequences described in Section 5.4, above. Alternatively, when utilized as part of assays such as those

described, below, in Section 5.3, "functionally equivalent" can refer to peptides capable of interacting with other cellular or extracellular molecules in a manner substantially similar to the way in which the corresponding portion of the endogenous differentially expressed or pathway gene product would. Functionally equivalent gene products therefore include naturally occurring differentially expressed or pathway gene products present in the same or different species. Functionally equivalent differentially expressed and/or pathway gene products also include gene products that retain at least one of the biological activities of the differentially expressed and/or pathway gene products described above; e.g., which are encoded by the coding sequences disclosed herein (as shown in FIGS. 2, 4A, 9, 12-15, 17, 22 and 24), contained in clones listed in Table 2, as deposited with the NRRL or ATCC. The functionally equivalent gene products of the pathway and/or differentially expressed genes of the invention also include gene products which are recognized by and bind to antibodies (polyclonal or monoclonal) directed against the differentially expressed and/or pathway gene products described above; e.g., which are encoded by the coding sequences disclosed herein (as shown in FIGS. 2, 4A, 9, 12-15, 17, 22 and 24), contained in clones listed in Table 2, as deposited with the NRRL or ATCC. The invention also includes degenerate variants of sequences (a) through (d).

The invention encompasses the following nucleotides, host cells expressing such nucleotides and the expression products of such nucleotides: (a) nucleotides that encode a mammalian differentially expressed and/or pathway gene product including, but not limited to a human and murine 10, 54, 57, 105, 106, 161 and 200 gene product; (b) nucleotides that encode portions of differentially expressed and/or pathway gene product that corresponds to its functional domains, and the polypeptide products encoded by such nucleotide sequences, and in which, in the case of receptor-type gene products, such domains include, but are not limited

to extracellular domains (ECD), transmembrane domains (TM) and cytoplasmic domains (CD); (c) nucleotides that encode mutants of a differentially expressed and/or pathway gene, product, in which all or part of one of its domains is
5 deleted or altered, and which, in the case of receptor-type gene products, such mutants include, but are not limited to, soluble receptors in which all or a portion of the TM is deleted, and nonfunctional receptors in which all or a portion of CD is deleted; and (d) nucleotides that encode
10 fusion proteins containing a differentially expressed and/or pathway gene product or one of its domains fused to another polypeptide.

The nucleotide sequences of the invention further include nucleotide sequences corresponding to the nucleotide
15 sequences of (a)-(d) above wherein one or more of the exons, or fragments thereof, have been deleted.

The nucleotide sequences of the invention still further include nucleotide sequences that have at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more nucleotide sequence
20 identity to the nucleotide sequences of (a)-(d) above. The nucleotide sequences of the invention also include nucleotide sequences that encode polypeptides having at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or higher amino acid sequence identity to the polypeptides encoded by the nucleotide
25 sequences of (a)-(d) above.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic
30 acid sequence for optimal alignment with a second amino acid or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue
35 or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function

of the number of identical positions shared by the sequences (i.e., % identity = # of identical overlapping positions/total # of positions x 100%). In one embodiment, the two sequences are the same length.

- 5 The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. U.S.A.* 87:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al., 1990, *J. Mol. Biol.* 215:403-0. BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, e.g., for score=100, wordlength=12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the present invention. BLAST protein searches can be performed with the XBLAST program parameters set, e.g., to score=50, wordlength=3 to obtain amino acid sequences homologous to a protein molecule of the present invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., 1997, *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-BLAST can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., of XBLAST and NBLAST) can be used (see, e.g., <http://www.ncbi.nlm.nih.gov>). Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, (1988) *CABIOS* 4:11-17. Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

- 5 The invention also includes nucleic acid molecules, preferably DNA molecules, that hybridize to, and are therefore the complements of, the DNA sequences (a) through (d), in the preceding paragraph. Such hybridization conditions can be highly stringent or less highly stringent,
- 10 as described above. The nucleic acid molecules of the invention that hybridize to the above described DNA sequences include oligodeoxyoligonucleotides ("oligos") which hybridize under highly stringent or stringent conditions to the DNA sequences (a) through (d) in the preceding paragraph. In
- 15 general, for oligos between 14 and 70 nucleotides in length the melting temperature (T_m) is calculated using the formula: $T_m(^{\circ}\text{C}) = 81.5 + 16.6(\log[\text{monovalent cations (molar)}] + 0.41(\% \text{ G+C}) - (500/N))$, where N is the length of the probe. If the hybridization is carried out in a solution containing
- 20 formamide, the melting temperature may be calculated using the equation: $T_m(^{\circ}\text{C}) = 81.5 + 16.6(\log[\text{monovalent cations (molar)}] + 0.41(\% \text{ G+C}) - (0.61\% \text{ formamide}) - (500/N))$ where N is the length of the probe. In general, hybridization is carried out at about 20-25 degrees below T_m (for DNA-DNA
- 25 hybrids) or about 10-15 degrees below T_m (for RNA-DNA hybrids). Other exemplary highly stringent conditions may refer, e.g., to washing in 6xSSC/0.05% sodium pyrophosphate at 37°C (for 14-base oligos), 48°C (for 17-base oligos), 55°C (for 20-base oligos), and 60°C (for 23-base oligos).
- 30 These nucleic acid molecules can encode or act as target gene antisense molecules, useful, for example, in target gene regulation and/or as antisense primers in amplification reactions of target, fingerprint, and/or pathway gene nucleic acid sequences. Further, such sequences can be used as part
- 35 of ribozyme and/or triple helix sequences, also useful for target gene regulation. Still further, such molecules can be used as components of diagnostic methods whereby the presence

of, or predisposition to, an immune disorder, e.g., TH cell subpopulation-related disorder, can be detected.

Fragments of the differentially expressed and pathway genes of the invention can be at least 10 nucleotides in 5 length. In alternative embodiments, the fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000 or more contiguous nucleotides in length. Alternatively, the fragments can comprise sequences that encode at least 10, 20, 10 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450 or more contiguous amino acid residues of the differentially expressed and pathway gene products. Fragments of the differentially expressed and pathway nucleic acid molecules of the invention can also refer to exons or introns of the above described 15 nucleic acid molecules, as well as portions of the coding regions of such nucleic acid molecules that encode function domains such as extracellular domains (ECD), transmembrane domains (TM) and cytoplasmic domains (CD).

The invention also encompasses (a) DNA vectors that 20 contain any of the foregoing coding sequences and/or their complements (i.e., antisense); (b) DNA expression vectors that contain any of the foregoing coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences; and (c) genetically 25 engineered host cells that contain any of the foregoing coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences in the host cell. As used herein, regulatory elements include but are not limited to inducible and non-inducible 30 promoters, enhancers, operators and other elements known to those skilled in the art that drive and regulate expression. Such regulatory elements include but are not limited to the cytomegalovirus hCMV immediate early gene, the early or late promoters of SV40 adenovirus, the lac system, the trp system, 35 the TAC system, the TRC system, the major operator and promoter regions of phage A, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the

promoters of acid phosphatase, and the promoters of the yeast α -mating factors. The invention includes fragments of any of the DNA sequences disclosed herein.

In addition to the gene sequences described above,
5 homologs of these gene sequences and/or full length coding sequences of these genes, as can be present in the same or other species, can be identified and isolated, without undue experimentation, by molecular biological techniques well known in the art. Further, there can exist genes at other
10 genetic loci within the genome of the same species that encode proteins which have extensive homology to one or more domains of such gene products. These genes can also be identified via similar techniques.

For example, the isolated differentially expressed gene
15 sequence can be labeled and used to screen a cDNA library constructed from mRNA obtained from the organism of interest. Hybridization conditions should be of a lower stringency when the cDNA library was derived from an organism different from the type of organism from which the labeled sequence was
20 derived. cDNA screening can also identify clones derived from alternatively spliced transcripts in the same or different species. Alternatively, the labeled fragment can be used to screen a genomic library derived from the organism of interest, again, using appropriately stringent conditions.
25 Low stringency conditions will be well known to those of skill in the art, and will vary predictably depending on the specific organisms from which the library and the labeled sequences are derived. For guidance regarding such conditions see, for example, Sambrook et al., 1989, Molecular
30 Cloning, A Laboratory Manual, Cold Springs Harbor Press, N.Y.; and Ausubel et al., 1989, Current Protocols in Molecular Biology, (Green Publishing Associates and Wiley Interscience, N.Y.).

Further, a previously unknown differentially expressed
35 or pathway gene-type sequence can be isolated by performing PCR using two degenerate oligonucleotide primer pools designed on the basis of amino acid sequences within the gene

of interest. The template for the reaction can be cDNA obtained by reverse transcription of mRNA prepared from human or non-human cell lines or tissue known or suspected to express a differentially expressed or pathway gene allele.

- 5 The PCR product can be subcloned and sequenced to insure that the amplified sequences represent the sequences of a differentially expressed or pathway gene-like nucleic acid sequence.

The PCR fragment can then be used to isolate a full
10 length cDNA clone by a variety of methods. For example, the amplified fragment can be used to screen a bacteriophage cDNA library. Alternatively, the labeled fragment can be used to screen a genomic library.

PCR technology can also be utilized to isolate full
15 length cDNA sequences. For example, RNA can be isolated, following standard procedures, from an appropriate cellular or tissue source. A reverse transcription reaction can be performed on the RNA using an oligonucleotide primer specific for the most 5' end of the amplified fragment for the priming
20 of first strand synthesis. The resulting RNA/DNA hybrid can then be "tailed" with guanines using a standard terminal transferase reaction, the hybrid can be digested with RNAase H, and second strand synthesis can then be primed with a poly-C primer. Thus, cDNA sequences upstream of the
25 amplified fragment can easily be isolated. For a review of cloning strategies which can be used, see e.g., Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Springs Harbor Press, N.Y.; and Ausubel et al., 1989, Current Protocols in Molecular Biology, (Green Publishing Associates
30 and Wiley Interscience, N.Y.).

As will be appreciated by those skilled in the art, DNA sequence polymorphisms of a differentially expressed or pathway gene identified by the methods of the present invention will exist within a population of individual
35 organisms (e.g., within a human population). Such polymorphisms may exist, for example, among individuals within a population due to natural allelic variation. Such

polymorphisms include ones that lead to changes in amino acid sequence. An allele is one of a group of genes which occurs alternatively at a given genetic locus. Accordingly, as used herein, an "allelic variant" refers to a nucleotide sequence
5 which occurs at a given locus or to a gene product encoded by the nucleotide sequence. Natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of a given gene.

Alternative alleles or allelic variants can be
10 identified by sequencing the gene of interest in a number of different individuals. This can be readily carried out by using hybridization probes to identify the same genetic locus in a variety of individuals. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules
15 comprising an open reading frame encoding a polypeptide of the invention. The term can further include nucleic acid molecules comprising upstream and/or exon/intron sequences and structure.

With respect to allelic variant of the differentially
20 expressed and pathway genes and gene products of the present invention, any and all nucleotide variations and/or amino acid polymorphisms or variations that are the result of natural allelic variation of the differentially expressed pathway genes and/or gene products are intended to be within
25 the scope of the present invention. Such allelic variants include, but are not limited to, ones that do not alter the functional activity of a differentially expressed or pathway gene product of the invention. Variants also include, but are not limited to "mutant alleles." As used herein, a
30 "mutant allele" of a differentially expressed or pathway gene or gene product of the invention is an allelic variant which does alter the functional activity of the differentially expressed or pathway gene product encoded by that gene.

In cases where the differentially expressed or pathway
35 gene identified is the normal, or wild type, gene, this gene can be used to isolate mutant alleles of the gene. Such an isolation is preferable in processes and disorders which are

known or suspected to have a genetic basis. Mutant alleles can be isolated from individuals either known or suspected to have a genotype which contributes to TH cell subpopulation-disorder related symptoms. Mutant alleles and mutant allele products can then be utilized in the therapeutic and diagnostic assay systems described below.

A cDNA of a mutant gene can be isolated, for example, by using PCR, a technique which is well known to those of skill in the art. In this case, the first cDNA strand can be synthesized by hybridizing a oligo-dT oligonucleotide to mRNA isolated from tissue known to, or suspected of, being expressed in an individual putatively carrying the mutant allele, and by extending the new strand with reverse transcriptase. The second strand of the cDNA is then synthesized using an oligonucleotide that hybridizes specifically to the 5' end of the normal gene. Using these two primers, the product is then amplified via PCR, cloned into a suitable vector, and subjected to DNA sequence analysis through methods well known to those of skill in the art. By comparing the DNA sequence of the mutant gene to that of the normal gene, the mutation(s) responsible for the loss or alteration of function of the mutant gene product can be ascertained.

Alternatively, a genomic or cDNA library can be constructed and screened using DNA or RNA, respectively, from a tissue known to or suspected of expressing the gene of interest in an individual suspected of or known to carry the mutant allele. The normal gene or any suitable fragment thereof can then be labeled and used as a probe to identify the corresponding mutant allele in the library. The clone containing this gene can then be purified through methods routinely practiced in the art, and subjected to sequence analysis as described, above, in this Section.

Additionally, an expression library can be constructed utilizing DNA isolated from or cDNA synthesized from a tissue known to or suspected of expressing the gene of interest in an individual suspected of or known to carry the mutant

allele. In this manner, gene products made by the putatively mutant tissue can be expressed and screened using standard antibody screening techniques in conjunction with antibodies raised against the normal gene product, as described, below, 5 in Section 5.6. (For screening techniques, see, for example, Harlow, E. and Lane, eds., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor.) In cases where the mutation results in an expressed gene product with altered function (e.g., as a result of a missense 10 mutation), a polyclonal set of antibodies are likely to cross-react with the mutant gene product. Library clones detected via their reaction with such labeled antibodies can be purified and subjected to sequence analysis as described in this Section, above.

15 Other allelic variants and/or mutant variants of the differentially expressed and pathway genes of the invention include single nucleotide polymorphisms (SNPs), including biallelic SNPs or biallelic markers which have two alleles, both of which are present at a fairly high frequency in a 20 population of organisms. Conventional techniques for detecting SNPs include, e.g., conventional dot blot analysis, single stranded conformational polymorphism (SSCP) analysis (see, e.g., Orita et al., 1989, *Proc. Natl. Acad. Sci. USA* 86:2766-2770), denaturing gradient gel electrophoresis 25 (DGGE), heteroduplex analysis, mismatch cleavage detection, and other routine techniques well known in the art (see, e.g., Sheffield et al., 1989, *Proc. Natl. Acad. Sci.* 86:5855-5892; Grompe, 1993, *Nature Genetics* 5:111-117).

Alternative, preferred methods of detecting and mapping SNPs 30 involve microsequencing techniques wherein an SNP site in a target DNA is detected by a single nucleotide primer extension reaction (see, e.g., Goelet et al., PCT Publication No. WO 92/15712; Mundy, U.S. Patent No. 4,656,127; Vary and Diamond, U.S. Patent No. 4,851,331; Cohen et al., PCT 35 Publication No. WO 91/02087; Chee et al., PCT Publication No. WO 95/11995; Landegren et al., 1988, *Science* 241:1077-1080; Nicerson et al., 1990, *Proc. Natl. Acad. Sci. U.S.A.*

87:9823-8927; Pastinen et al., 1997, *Genome Res.* 7:606-614; Pastinen et al., 1996, *Clin. Chem.* 42:1391-1397; Jalanko et al., 1992, *Clin. Chem* 38:39-43; Shumaker et al., 1996, *Hum. Mutation* 7:346-354; Caskey et al., PCT Publication No. 5 95/00669).

5.5. DIFFERENTIALLY EXPRESSED AND PATHWAY GENE PRODUCTS

Differentially expressed and pathway gene products include those proteins encoded by the differentially
10 expressed and pathway genes corresponding to the gene sequences described in Section 5.4, above, as, for example, the peptides listed in FIGS. 9, 17, 22 and 24.

In addition, differentially expressed and pathway gene products can include proteins that represent functionally
15 equivalent gene products. Such gene products include, but are not limited to natural variants of the peptides listed in FIGS. 9, 17, 22 and 24. Such an equivalent differentially expressed or pathway gene product can contain deletions, additions or substitutions of amino acid residues within the
20 amino acid sequence encoded by the differentially expressed or pathway gene sequences described, above, in Section 5.4, but which result in a silent change, thus producing a functionally equivalent differentially expressed or pathway gene product. Amino acid substitutions can be made on the
25 basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and
30 methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid.
35 "Functionally equivalent", as utilized herein, refers to a protein capable of exhibiting a substantially similar in vivo activity as the endogenous differentially expressed or

pathway gene products encoded by the differentially expressed or pathway gene sequences described in Section 5.4, above.

Alternatively, when utilized as part of assays such as those described, below, in Section 5.3, "functionally equivalent"

5 can refer to peptides capable of interacting with other cellular or extracellular molecules in a manner substantially similar to the way in which the corresponding portion of the endogenous differentially expressed or pathway gene product would.

10 Peptides corresponding to one or more domains of the differentially expressed or pathway gene products (e.g., TM, ECD or CD), truncated or deleted differentially expressed or pathway gene products (e.g., in the case of receptor-type gene products, proteins in which the full length
15 differentially expressed or pathway gene products, a differentially expressed or pathway gene peptide or truncated differentially expressed or pathway gene product is fused to an unrelated protein are also within the scope of the invention and can be designed on the basis of the
20 differentially expressed or pathway gene nucleotide and amino acid sequences disclosed in this Section and in Section 5.4, above. Such fusion proteins include but are not limited to IgFC fusions which stabilize the differentially expressed or pathway gene and prolong half-life in vivo; or fusions to any
25 amino acid sequence that allows the fusion protein to be anchored to the cell membrane, allowing peptides to be exhibited on the cell surface; or fusions to an enzyme, fluorescent protein, or luminescent protein which provide a marker function.

30 Other mutations to the differentially expressed or pathway gene product coding sequence can be made to generate polypeptides that are better suited for expression, scale up, etc. in the host cells chosen. For example, cysteine residues can be deleted or substituted with another amino
35 acid in order to eliminate disulfide bridges; in the case of secreted or transmembrane proteins, N-linked glycosylation sites can be altered or eliminated to achieve, for example,

expression of a homogeneous product that is more easily recovered and purified from yeast hosts which are known to hyperglycosylate N-linked sites. To this end, a variety of amino acid substitutions at one or both of the first or third amino acid positions of any one or more of the glycosylation recognition sequences (N-X-S or N-X-T), and/or an amino acid deletion at the second position of any one or more such recognition sequences will prevent glycosylation of the protein at the modified tripeptide sequence. (See, e.g., Miyajima et al., 1986, EMBO J. 5(6):1193-1197).

The differentially expressed or pathway gene products of the invention comprise at least as many contiguous amino acid residues as necessary to represent an epitope fragment (that is to be recognized by an antibody directed to the differentially expressed or pathway gene product). For example, such protein fragments or peptides can comprise at least about 8 contiguous amino acid residues from a full length differentially expressed or pathway gene product. In alternative embodiments, the protein fragments and peptides of the invention can comprise about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, or more contiguous amino acid residues of a differentially expressed or pathway gene product.

Peptides and/or proteins corresponding to one or more domains of the differentially expressed or pathway gene products are also within the scope of the present invention. Further, fusion proteins in which a differentially expressed or pathway gene product, or a portion thereof such as a truncated differentially expressed or pathway gene product or a domain of a differentially expressed or pathway gene product, is fused to an unrelated protein are within the scope of the present invention. Such proteins and peptides can be designed on the basis of the differentially expressed and pathway gene sequences disclosed in Section 5.1 above, and/or on the basis of the differentially expressed and pathway gene product sequences disclosed in this section.

Fusion proteins of the invention include, but are not limited to, IgFc fusion proteins which are useful, e.g., to stabilize the differentially expressed or pathway gene product such that the gene product has a prolonged half-life in vivo, as well as, e.g., fusions to any amino acid sequence that allows the fusion protein to be anchored to the cell membrane, fusions to an enzyme, fluorescent protein, luminescent protein, or a flag epitope protein or peptide which may be used to provide a marker function.

10 Finally, the differentially expressed and pathway gene products of the present invention also include amino acid sequences encoded by the differentially expressed or pathway genes of the invention wherein domains encoded by one or more exons of the cDNA sequences of those genes, or fragments
15 thereof, have been deleted. The differentially expressed and pathway gene products of the invention can still further comprise post translational modifications, including, but not limited to glycosylations, acetylations, and myrisalations.

The differentially expressed or pathway gene products
20 can be produced by synthetic techniques or via recombinant DNA technology using techniques well known in the art. Thus, methods for preparing the differentially expressed or pathway gene polypeptides and peptides of the invention are described herein. First, the polypeptides and peptides of the
25 invention can be synthesized or prepared by techniques well known in the art. See, for example, Creighton, 1983, "Proteins: Structures and Molecular Principles", W.H. Freeman and Co., N.Y., which is incorporated herein by reference in its entirety. Peptides can, for example, be
30 synthesized on a solid support or in solution.

Alternatively, recombinant DNA methods which are well known to those skilled in the art can be used to construct expression vectors containing differentially expressed or pathway gene protein coding sequences and appropriate
35 transcriptional/translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques and in vivo recombination/genetic

recombination. See, for example, the techniques described in Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. which is incorporated by reference herein in their entirety, 5 and Ausubel, 1989, supra. Alternatively, RNA capable of encoding differentially expressed or pathway gene protein sequences can be chemically synthesized using, for example, synthesizers. See, for example, the techniques described in "Oligonucleotide Synthesis", 1984, Gait, M.J. ed., IRL Press, 10 Oxford, which is incorporated by reference herein in its entirety.

A variety of host-expression vector systems can be utilized to express the differentially expressed or pathway gene coding sequences of the invention. Such host-expression 15 systems represent vehicles by which the coding sequences of interest can be produced and subsequently purified, but also represent cells which can, when transformed or transfected with the appropriate nucleotide coding sequences, exhibit the differentially expressed or pathway gene protein of the 20 invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., E. coli, B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing differentially expressed or pathway gene protein coding sequences; yeast 25 (e.g., Saccharomyces, Pichia) transformed with recombinant yeast expression vectors containing the differentially expressed or pathway gene protein coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the differentially 30 expressed or pathway gene protein coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing differentially 35 expressed or pathway gene protein coding sequences; or mammalian cell systems (e.g. COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing

promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

- 5 In bacterial systems, a number of expression vectors can be advantageously selected depending upon the use intended for the differentially expressed or pathway gene protein being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of antibodies
10 or to screen peptide libraries, for example, vectors which direct the expression of high levels of fusion protein products that are readily purified can be desirable. Such vectors include, but are not limited, to the E. coli expression vector pUR278 (Ruther et al., 1983, EMBO J.
15 2:1791), in which the differentially expressed or pathway gene protein coding sequence can be ligated individually into the vector in frame with the lacZ coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke & Schuster,
20 1989, J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors can also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-
25 agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene protein can be released from the GST moiety.
- 30 In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in Spodoptera frugiperda cells. The differentially expressed or pathway gene coding sequence can be cloned individually into non-essential
35 regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of differentially

expressed or pathway gene coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These
5 recombinant viruses are then used to infect Spodoptera frugiperda cells in which the inserted gene is expressed, (e.g., see Smith et al., 1983, J. Virol. 46:584; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral-based
10 expression systems can be utilized. In cases where an adenovirus is used as an expression vector, the differentially expressed or pathway gene coding sequence of interest can be ligated to an adenovirus transcription/translation control complex, e.g., the late
15 promoter and tripartite leader sequence. This chimeric gene can then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing
20 differentially expressed or pathway gene protein in infected hosts, (e.g., See Logan & Shenk, 1984, Proc. Natl. Acad. Sci. USA 81:3655-3659). Specific initiation signals can also be required for efficient translation of inserted differentially expressed or pathway gene coding sequences. These signals
25 include the ATG initiation codon and adjacent sequences. In cases where an entire differentially expressed or pathway gene, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals can be
30 needed. However, in cases where only a portion of the differentially expressed or pathway gene coding sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the
35 reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of

a variety of origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., 1987, Methods in
5 Enzymol. 153:516-544).

In addition, a host cell strain can be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation)
10 and processing (e.g., cleavage) of protein products can be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. Appropriate cell lines or host systems can be chosen to
15 ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product can be used. Such mammalian host cells include
20 but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, etc.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the differentially expressed or
25 pathway gene protein can be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators,
30 polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells can be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers
35 resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell

lines. This method can advantageously be used to engineer cell lines which express the differentially expressed or pathway gene protein. Such engineered cell lines can be particularly useful in screening and evaluation of compounds that affect the endogenous activity of the differentially expressed or pathway gene protein.

A number of selection systems can be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, et al., 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48:2026), and adenine phosphoribosyltransferase (Lowy, et al., 1980, Cell 22:817) genes can be employed in tk⁻, hgp⁺ or ap⁺ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, et al., 1980, Natl. Acad. Sci. USA 77:3567; O'Hare, et al., 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, et al., 1981, J. Mol. Biol. 150:1); and hyg⁺, which confers resistance to hygromycin (Santerre, et al., 1984, Gene 30:147) genes.

Alternatively, any fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cells lines (Janknecht, et al., 1991, Proc. Natl. Acad. Sci. USA 88: 8972-8976). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni²⁺-nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

When used as a component in assay systems such as those described herein, the differentially expressed or pathway gene protein can be labeled, either directly or indirectly, to facilitate detection of a complex formed between the
5 differentially expressed or pathway gene protein and a test substance. Any of a variety of suitable labeling systems can be used including but not limited to radioisotopes such as ^{125}I ; enzyme labelling systems that generate a detectable colorimetric signal or light when exposed to substrate; and
10 fluorescent labels.

Indirect labeling involves the use of a protein, such as a labeled antibody, which specifically binds to either a differentially expressed or pathway gene product. Such antibodies include but are not limited to polyclonal,
15 monoclonal, chimeric, single chain, Fab fragments and fragments produced by an Fab expression library.

Where recombinant DNA technology is used to produce the differentially expressed or pathway gene protein for such assay systems, it can be advantageous to engineer fusion
20 proteins that can facilitate labeling (either direct or indirect), immobilization, solubility and/or detection.

Fusion proteins, which can facilitate solubility and/or expression, and can increase the blood half-life of the protein, can include, but are not limited to soluble Ig-
25 tailed fusion proteins. Methods for engineering such soluble Ig-tailed fusion proteins are well known to those of skill in the art. See, for example U.S. Patent No. 5,116,964, which is incorporated herein by reference in its entirety.

Further, in addition to the Ig-region encoded by the IgG1
30 vector, the Fc portion of the Ig region utilized can be modified, by amino acid substitutions, to reduce complement activation and Fc binding. (See, e.g., European Patent No. 239400 B1, August 3, 1994).

Among the soluble Ig-tailed fusion proteins which can be
35 produced are soluble Ig-tailed fusion proteins containing 103 gene products, 200 gene products or 10 gene products. The 103 gene product or 200 gene contained within such fusion

proteins can comprise, respectively, for example, the 103 gene extracellular or secreted domain or portions, preferably ligand-binding portions, thereof, or the 200 gene extracellular domain or portions, preferably ligand-binding portions, thereof. The 10 gene product contained within such fusion proteins can comprise, for example, one or more of the extracellular domains or portions, preferably ligand-binding portions, of the seven transmembrane domain sequence motif.

The amino acid sequences of the secreted and membrane bound forms of the murine 103 gene products are known. Further, the amino acid sequence of a soluble human 103 gene product is known. (See, for example, Klemenz, R. et al., 1989, Proc. Natl. Acad. Sci. USA 86:5708-5712; Tominaga, S., 1989, FEBS Lett. 258:301-301; Werenskiold, A.K. et al., 1989, Mol. Cell. Biol. 9:5207-5214; Tominaga, S. et al., 1992, Biochem. Biophys. Acta. 1171:215-218; Werenskiold, A.K., 1992, Eur. J. Biochem. 204:1041-1047; Yanagisawa, K. et al., 1993, FEBS Lett. 318:83-87; Bergers, G. et al., 1994, EMBO J. 13:1176-1188; and Kumar, S. 1997, Biochem Biophys Res Commun 235:474-478.)

Further, as indicated in FIG. 4B, the amino acid residues which delineate the extracellular, transmembrane and cytoplasmic domains of the murine 103 gene products are also known. Still further, the amino acid sequences of murine and human 103 gene products are listed in SEQ ID NOS: 39 (murine full length, transmembrane 103 gene product), 41 (murine extracellular domain of the full length, transmembrane product, plus amino terminal signal peptide), 43 (murine intracellular domain of the full length, transmembrane product), 48 (murine transmembrane domain of the full length, transmembrane product), 47 (murine secreted 103 gene product), and 45 (human secreted/extracellular 103 gene product domain, plus amino terminal signal peptide).

The murine 103 gene encodes two mRNA products, a 2.5 Kb transcript and a 4.5 Kb transcript, which correspond to the soluble and membrane forms of the 103 protein (Kumar, S. 1997, Biochem Biophys Res Commun 235:474-478). In contrast,

the human 103 gene encodes three mRNA products, an approximately 4.2 Kb transcript, an approximately 2.5 Kb transcript, and an approximately 1.4 Kb transcript (Kumar, S. 1997, Biochem Biophys Res Commun 235:474-478). Nucleotide sequences encoding 103 gene products are listed herein at SEQ ID NOS: 38 (nucleotide sequence encoding murine full length, transmembrane 103 gene product), 40 (nucleotide sequence encoding extracellular domain of the murine 103 transmembrane gene product, plus amino terminal signal peptide), 42 (nucleotide sequence encoding the intracellular domain of the murine 103 transmembrane gene product), 46 (nucleotide sequence encoding the transmembrane domain of the murine 103 transmembrane gene product), 49 (nucleotide sequence encoding the secreted murine 103 gene product), and 44 (nucleotide sequence encoding human secreted/extracellular 103 gene product domain, plus amino terminal signal peptide). Murine and human 103 amino acid and nucleotide sequences are also depicted in FIG. 4C, FIG. 4D, FIG. 4E, FIG. 4F, FIG. 4G, and FIG. 4H. The nucleotide sequences of the 4.2 Kb and 2.5 Kb human 103 gene transcripts, as well as the amino acid sequences encoded by these transcripts, can be obtained utilizing standard techniques well known to those of skill in the art, including, e.g., techniques such as those discussed herein.

25 Therefore, by utilizing well known techniques, one of skill in the art would readily be capable of producing such soluble Ig-tailed 103 gene product fusion proteins. The Example presented below, in Section 10, below, describes the construction of a 103 gene product-Ig fusion protein.

30 The signal sequence, extracellular, transmembrane and cytoplasmic domains of both the murine and human 200 gene products have been elucidated and can be utilized in, for example, the construction of 200 gene product-Ig fusion proteins. Specifically, the 280 amino acid murine 200 gene product (FIG. 17; SEQ ID NO:10) contains a signal sequence from approximately amino acid residue 1 to approximately amino acid residue 20, an extracellular domain from

approximately amino acid residue 21 to approximately amino acid residue 192, a transmembrane domain from approximately amino acid residue 193 to amino acid residue 214, and a cytoplasmic domain from approximately amino acid residue 215 to amino acid residue 280. Further, the 301 amino acid human 200 gene product (FIG. 24; SEQ. ID. NO: 24) contains a signal sequence from amino acid residue 1 to approximately 20, a mature extracellular domain from approximately amino acid residue 21 to 200, a transmembrane domain from approximately amino acid residue 201-224 and a cytoplasmic domain from approximately amino acid residue 225 to 301. Given the elucidation of these domains, one of skill in the art would readily be capable of producing soluble Ig-tailed 200 gene product fusion proteins. The Example presented, below, in Section 10 describes the construction of murine and human 200 gene product-Ig fusion proteins.

The 338 amino acid residue 10 gene product (FIG.9, SEQ ID NO:9) extracellular domains include 10 gene product amino acid residues from approximately amino acid residue 1 to 19, approximately amino acid residue 74 to 87, approximately amino acid residue 153 to 187 and approximately amino acid residue 254 to 272. Thus, such 10 gene product domain information can be used, in conjunction with well-known techniques, such that one of skill in the art can readily be capable of producing soluble Ig-tailed 10 gene fusion proteins comprising one or more 10 gene product extracellular domain regions and an Ig tail.

5.6. ANTIBODIES SPECIFIC FOR DIFFERENTIALLY EXPRESSED OR PATHWAY GENE PRODUCTS

Described herein are methods for the production of antibodies capable of specifically recognizing one or more differentially expressed or pathway gene product epitopes. Such antibodies can include, but are not limited to, polyclonal antibodies, monoclonal antibodies (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab')₂ fragments, fragments produced by a Fab

expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. The Ig tails of such antibodies can be modified to reduce complement activation and Fc binding. (See, for example, European 5 Patent No. 239400 B1, August 3, 1994).

Such antibodies can be used, for example, in the detection of a fingerprint, target, or pathway gene product in a biological sample, and can be used as part of diagnostic techniques. Alternatively, such antibodies can be utilized 10 as part of an immune disorder treatment method, as described, below, in Section 5.9. For example, the antibodies can be used to modulate target gene activity, can be used to modulate TH cell subpopulation differentiation, maintenance and/or effector function, or, in the case of antibodies 15 directed to cell surface epitopes, can be used to isolate a TH cell subpopulation of interest, for either depletion or augmentation purposes.

Such antibodies can also be utilized as part of a method for treatment of an ischemic disorder or injury, as described 20 in Section 5.10.3.1, below. For example, the antibodies can be used to block or inhibit activity of one or more of the gene products of the invention, thereby reducing or inhibiting repair of certain ischemic tissues, for example carcinogenic tumors.

25 For the production of antibodies to a differentially expressed or pathway gene, various host animals can be immunized by injection with a differentially expressed or pathway gene protein, or a portion thereof. Such host animals can include but are not limited to rabbits, mice, and 30 rats, to name but a few. Various adjuvants can be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, 35 polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human

adjuvants such as BCG (bacille Calmette-Guerin) and Corynebacterium parvum.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized
5 with an antigen, such as target gene product, or an antigenic functional derivative thereof. For the production of polyclonal antibodies, host animals such as those described above, can be immunized by injection with differentially expressed or pathway gene product supplemented
10 with adjuvants as also described above.

Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, can be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture. These
15 include, but are not limited to the hybridoma technique of Kohler and Milstein, (1975, Nature 256:495-497; and U.S. Patent No. 4,376,110), the human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today 4:72; Cole et al., 1983, Proc. Natl. Acad. Sci. USA 80:2026-2030), and the EBV-
20 hybridoma technique (Cole et al., 1985, Monoclonal Antibodies And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention can be cultivated in
25 vitro or in vivo. Production of high titers of mAbs in vivo makes this the presently preferred method of production.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature,
30 312:604-608; Takeda et al., 1985, Nature, 314:452-454; U.S. Patent No. 4,816,567) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a
35 molecule in which different portions are derived from different animal species, such as those having a variable

region derived from a murine mAb and a human immunoglobulin constant region.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, 5 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-546) and for making humanized monoclonal antibodies (U.S. Patent No. 5,225,539, which is incorporated herein by reference in its entirety) can be utilized to produce anti-
10 differentially expressed or anti-pathway gene product antibodies.

Antibody fragments which recognize specific epitopes can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')₂,
15 fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries can be constructed (Huse et al., 1989, Science, 246:1275-1281) to
20 allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Antibodies to the differentially expressed or pathway gene products can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" such gene products, using
25 techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, 1993, FASEB J 7(5):437-444; and Nissinoff, 1991, J. Immunol. 147(8):2429-2438). For example, in the case of receptor-type molecules (e.g., 10, 103 and 200 gene products) antibodies which bind to the ECD and
30 competitively inhibit the binding of ligand to the receptor can be used to generate anti-idiotypes that "mimic" the ECD and, therefore, bind and neutralize the ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens of TH cell
35 subpopulation-related disorders.

Production of antibodies directed against the extracellular domain of the 103 gene product are described in

Section 12, below. Also, production of antibodies directed against the extracellular domain of the 200 gene product are described in Section 13, below.

5 5.7. CELL-AND ANIMAL-BASED MODEL SYSTEMS

Described herein are cell- and animal-based systems which act as models for immune disorders and for models of TH cell subpopulation differentiation, maintenance, and/or effector function. These systems can be used in a variety of applications. For example, the animal-based model systems can be utilized to identify differentially expressed genes via the in vivo paradigm described, above, in Section 5.1.1.1. Cell- and animal-based model systems can also be used to further characterize differentially expressed and pathway genes, as described, above, in Section 5.3. Such further characterization can, for example, indicate that a differentially expressed gene is a target gene. Second, such assays can be utilized as part of screening strategies designed to identify compounds which are capable of ameliorating TH cell subpopulation-related disorder symptoms, as described, below. Thus, the animal- and cell-based models can be used to identify drugs, pharmaceuticals, therapies and interventions which can be effective in treating immune disorders such as TH cell subpopulation-related disorders. In addition, as described in detail, below, in Section 5.11.1, such animal models can be used to determine the LD₅₀ and the ED₅₀ in animal subjects, and such data can be used to determine the in vivo efficacy of potential immune disorder treatments.

30

5.7.1 ANIMAL-BASED SYSTEMS

Animal-based model systems of TH cell subpopulation-related disorders can include both non-recombinant animals as well as recombinantly engineered transgenic animals.

35 Animal models for TH cell subpopulation-related disorders can include, for example, genetic models. For example, such animal models can include Leishmania resistance

models, experimental allergic encephalomyelitis models and (BALB/c Cr x DBA/2Cr) F1 mice. These latter mice develop a fatal disseminated disease by systemic infection with virulent Candida albicans associated with strong TH2-like
5 responses. Additionally, well known mouse models for asthma can be utilized to study the amelioration of symptoms caused by a TH2-like response. (See, for example, Lukacs, N.W. et al., 1994, Am. J. Resp. Cell Mol. Biol. 10:526-532; Gavett, S.H. et al., 1994, Am. J. Resp. Cell Mol. Biol. 10:587-593.)
10 Further, the animal model, murine acquired immunodeficiency syndrome (MAIDS; Kanagawa, B. et al., 1993, Science 262:240; Makino, M. et al., 1990, J. Imm. 144:4347) can be used for such studies.

Alternatively, such well known animal models as SCIDhu
15 mice (see for example, Kaneshima, H. et al., 1994, Curr. Opin. Imm. 6:327-333) which represents an in vivo model of the human hematolymphoid system, can be utilized. Further, the RAG-2-deficient blastocyst complementation technique (Chen, J. et al., 1993, Proc. Natl. Acad. Sci. USA 90:4528-
20 4532; Shinkai, Y. et al., 1992, Cell 68:855-867) can be utilized to produce mice containing, for example, humanized lymphocytes and/or which express target gene sequences. Still further, targeting techniques directed specifically to T cells, for example, the technique of Gu et al. (Gu, H. et
25 al., 1994, Science 265:103-106) can be utilized to produce animals containing transgenes in only T cell populations.

Further, animal models such as the adoptive transfer model described, e.g., in Cohn, L. et al., 1997, J. Exp. Med. 186:1737-1747) and described and utilized in Section 12,
30 below, can be used. In such an animal system, aeroallergen provocation of TH1 or TH2 recipient mice results in TH effector cell migration to the airways and is associated with an intense neutrophilic (TH1) and eosinophilic (TH2) lung mucosal inflammatory response. The animal model represents
35 an accepted model for the TH2-like disorder asthma.

Animal models exhibiting TH cell subpopulation-related disorder-like symptoms can be engineered by utilizing, for

example, target gene sequences such as those described, above, in Section 5.4, in conjunction with techniques for producing transgenic animals that are well known to those of skill in the art. For example, target gene sequences can be introduced into, and overexpressed and/or misexpressed in, the genome of the animal of interest, or, if endogenous target gene sequences are present, they can either be overexpressed, misexpressed, or, alternatively, can be disrupted in order to underexpress or inactivate target gene expression. The construction and characterization of 200 gene and 103 gene transgenic animals is described in Section 11, below.

In order to overexpress or misexpress a target gene sequence, the coding portion of the target gene sequence can be ligated to a regulatory sequence which is capable of driving high level gene expression or expression in a cell type in which the gene is not normally expressed in the animal and/or cell type of interest. Such regulatory regions will be well known to those of skill in the art, and can be utilized in the absence of undue experimentation.

For underexpression of an endogenous target gene sequence, such a sequence can be isolated and engineered such that when reintroduced into the genome of the animal of interest, the endogenous target gene alleles will be inactivated. Preferably, the engineered target gene sequence is introduced via gene targeting such that the endogenous target sequence is disrupted upon integration of the engineered target gene sequence into the animal's genome. Gene targeting is discussed, below, in this Section.

Animals of any species, including, but not limited to, mice, rats, rabbits, guinea pigs, pigs, micro-pigs, goats, and non-human primates, e.g., baboons, squirrels, monkeys, and chimpanzees can be used to generate animal models of TH cell subpopulation-related disorders.

Any technique known in the art can be used to introduce a target gene transgene into animals to produce the founder lines of transgenic animals. Such techniques include, but

are not limited to pronuclear microinjection (Hoppe, P.C. and Wagner, T.E., 1989, U.S. Pat. No. 4,873,191); retrovirus mediated gene transfer into germ lines (Van der Putten et al., 1985, Proc. Natl. Acad. Sci., USA 82:6148-6152); gene
5 targeting in embryonic stem cells (Thompson et al., 1989, Cell 56:313-321); electroporation of embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814); and sperm-mediated gene transfer (Lavitrano et al., 1989, Cell 57:717-723); etc. For a review
10 Intl. Rev. Cytol. 115:171-229, which is incorporated by reference herein in its entirety.

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their
15 cells, i.e., mosaic animals. (See, for example, techniques described by Jakobovits, 1994, Curr. Biol. 4:761-763.) The transgene can be integrated as a single transgene or in concatamers, e.g., head-to-head tandems or head-to-tail tandems. The transgene can also be selectively introduced
20 into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko, M. et al., 1992, Proc. Natl. Acad. Sci. USA 89:6232-6236). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of
25 interest, and will be apparent to those of skill in the art.

When it is desired that the target gene transgene be integrated into the chromosomal site of the endogenous target gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some
30 nucleotide sequences homologous to the endogenous target gene of interest are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of, the nucleotide sequence of the endogenous target gene. The transgene can also be
35 selectively introduced into a particular cell type, thus inactivating the endogenous gene of interest in only that cell type, by following, for example, the teaching of Gu et

al. (Gu, H. et al., 1994, Science 265:103-106). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

5 Once transgenic animals have been generated, the expression of the recombinant target gene and protein can be assayed utilizing standard techniques. Initial screening can be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to assay whether integration of the
10 transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals can also be assessed using techniques which include but are not limited to Northern blot analysis of tissue samples obtained from the animal, in situ hybridization analysis, and RT-PCR.
15 Samples of target gene-expressing tissue, can also be evaluated immunocytochemically using antibodies specific for the target gene transgene gene product of interest.

 The target gene transgenic animals that express target gene mRNA or target gene transgene peptide (detected
20 immunocytochemically, using antibodies directed against target gene product epitopes) at easily detectable levels can then be further evaluated to identify those animals which display characteristic TH cell subpopulation-related disorder-like symptoms, or exhibit characteristic TH cell
25 subpopulation differentiation phenotypes. TH1-like-related disorder symptoms can include, for example, those associated with chronic inflammatory diseases and disorders, such as Crohn's disease, reactive arthritis, including Lyme disease, insulin-dependent diabetes, organ-specific autoimmunity,
30 including multiple sclerosis, Hashimoto's thyroiditis and Grave's disease, contact dermatitis, psoriasis, graft rejection, graft versus host disease and sarcoidosis. TH2-like-related disorder symptoms can include, those associated with atopic conditions, such as asthma and allergy, including
35 allergic rhinitis, gastrointestinal allergies, including food allergies, eosinophilia, conjunctivitis, glomerular nephritis, certain pathogen susceptibilities such as

helminthic (e.g., leishmaniasis) and certain viral infections, including HIV, and bacterial infections, including tuberculosis and lepromatous leprosy.

Additionally, specific cell types within the transgenic
5 animals can be analyzed and assayed for cellular phenotypes characteristic of TH cell subpopulation-related disorders. Such cellular phenotypes can include, for example, differential cytokine expression characteristic of the TH cell subpopulation of interest. Further, such cellular
10 phenotypes can include an assessment of a particular cell type's fingerprint pattern of expression and its comparison to known fingerprint expression profiles of the particular cell type in animals exhibiting specific TH cell subpopulation-related disorders. Such transgenic animals
15 serve as suitable model systems for TH cell-related disorders.

Once target gene transgenic founder animals are produced (i.e., those animals which express target gene proteins in cells or tissues of interest, and which, preferably, exhibit
20 symptoms of TH cell subpopulation-related disorders), they can be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include but are not limited to: outbreeding of founder animals with more than one integration site in order
25 to establish separate lines; inbreeding of separate lines in order to produce compound target gene transgenics that express the target gene transgene of interest at higher levels because of the effects of additive expression of each target gene transgene; crossing of heterozygous transgenic
30 animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the possible need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; breeding animals to
35 different inbred genetic backgrounds so as to examine effects of modifying alleles on expression of the target gene transgene and the development of TH cell subpopulation-

related disorder-like symptoms. One such approach is to cross the target gene transgenic founder animals with a wild type strain to produce an F1 generation that exhibits TH cell subpopulation-related disorder-like symptoms, such as those
5 described above. The F1 generation can then be inbred in order to develop a homozygous line, if it is found that homozygous target gene transgenic animals are viable.

5.7.2. CELL-BASED ASSAYS

10 Cells that contain and express target gene sequences which encode target gene protein, and, further, exhibit cellular phenotypes associated with a TH cell subpopulation-related disorder of interest, can be utilized to identify compounds that exhibit an ability to ameliorate TH cell
15 subpopulation-related disorder symptoms. Cellular phenotypes which can indicate an ability to ameliorate TH cell subpopulation-related disorder symptoms can include, for example, an inhibition or potentiation of cytokine or cell surface marker expression associated with the TH cell
20 subpopulation of interest, or, alternatively, an inhibition or potentiation of specific TH cell subpopulations.

Further, the fingerprint pattern of gene expression of cells of interest can be analyzed and compared to the normal, non-TH cell subpopulation-related disorder fingerprint
25 pattern. Those compounds which cause cells exhibiting TH cell subpopulation-related disorder-like cellular phenotypes to produce a fingerprint pattern more closely resembling a normal fingerprint pattern for the cell of interest can be considered candidates for further testing regarding an
30 ability to ameliorate TH cell subpopulation-related disorder symptoms.

Cells which can be utilized for such assays can, for example, include non-recombinant cell lines, such as Dorris, AE7, D10.G4, DAX, D1.1 and CDC25 cell lines. In addition,
35 purified primary naive T cells derived from either transgenic or non-transgenic strains can also be used.

Further, cells which can be used for such assays can also include recombinant, transgenic cell lines. For example, the TH cell subpopulation-related disorder animal models of the invention, discussed, above, in Section 5.7.1, can be used to generate, for example, TH1-like and/or TH2-like cell lines that can be used as cell culture models for the disorder of interest. While primary cultures derived from TH cell subpopulation-related disorder transgenic animals can be utilized, the generation of continuous cell lines is preferred. For examples of techniques which can be used to derive a continuous cell line from the transgenic animals, see Small et al., 1985, Mol. Cell Biol. 5:642-648.

Alternatively, cells of a cell type known to be involved in TH cell subpopulation-related disorders can be transfected with sequences capable of increasing or decreasing the amount of target gene expression within the cell. For example, target gene sequences can be introduced into, and overexpressed in, the genome of the cell of interest, or, if endogenous target gene sequences are present, they can either be overexpressed or, alternatively, can be disrupted in order to underexpress or inactivate target gene expression.

In order to overexpress a target gene sequence, the coding portion of the target gene sequence can be ligated to a regulatory sequence which is capable of driving gene expression in the cell type of interest. Such regulatory regions will be well known to those of skill in the art, and can be utilized in the absence of undue experimentation.

For underexpression of an endogenous target gene sequence, such a sequence can be isolated and engineered such that when reintroduced into the genome of the cell type of interest, the endogenous target gene alleles will be inactivated. Preferably, the engineered target gene sequence is introduced via gene targeting such that the endogenous target sequence is disrupted upon integration of the engineered target gene sequence into the cell's genome. Gene targeting is discussed, above, in Section 5.7.1.

Transfection of target gene sequence nucleic acid can be accomplished by utilizing standard techniques. See, for example, Ausubel, 1989, supra. Transfected cells should be evaluated for the presence of the recombinant target gene sequences, for expression and accumulation of target gene mRNA, and for the presence of recombinant target gene protein production. In instances wherein a decrease in target gene expression is desired, standard techniques can be used to demonstrate whether a decrease in endogenous target gene expression and/or in target gene product production is achieved.

Cells to be utilized can, for example, be stimulated or activated as, described e.g., in the Examples presented below.

15

5.8. SCREENING ASSAYS FOR COMPOUNDS THAT INTERACT WITH THE TARGET GENE PRODUCT

The following assays are designed to identify compounds that bind to target gene products, bind to other cellular proteins that interact with a target gene product, and to compounds that interfere with the interaction of the target gene product with other cellular proteins. For example, in the cases of 10, 103 and 200 gene products, which are or are predicted to be transmembrane receptor-type proteins, such techniques can identify ligands for such receptors. A compound which binds a 103 gene product (a 103 gene product ligand, for example) can act as the basis for amelioration of such TH2-like-specific disorders as asthma or allergy, given that gene 103 expression is TH2-specific. A 200 gene product ligand can, for example, act as the basis for amelioration of TH1-like-specific disorders. A 10 gene product ligand can, for example, act as the basis for amelioration of a wide range of T cell disorders, given the TH inducible nature of its gene expression pattern. Any such binding compound can act as a marker for the presence of TH cell subpopulations. For example, a compound which binds the 103 gene product can

act as a marker, for example, a diagnostic marker, for TH2 cells, e.g., for TH2 cell differentiation.

Compounds can include, but are not limited to, other cellular proteins. Further, such compounds can include, but
5 are not limited to, peptides such as, for example, soluble peptides, including, but not limited to, Ig-tailed fusion peptides, comprising extracellular portions of target gene product transmembrane receptors, and members of random peptide libraries (see, e.g., Lam, K.S. et al., 1991, Nature
10 354:82-84; Houghten, R. et al., 1991, Nature 354:84-86) made of D-and/or L-configuration amino acids, phosphopeptides (including but not limited to members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang, Z. et al., 1993, Cell 72:767-778), antibodies
15 (including, but not limited to polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and FAb, F(ab')₂ and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules. In the case of receptor-type
20 target molecules, such compounds can include organic molecules (e.g., peptidomimetics) that bind to the ECD and either mimic the activity triggered by the natural ligand (i.e., agonists); as well as peptides, antibodies or fragments thereof, and other organic compounds that mimic the
25 ECD (or a portion thereof) and bind to a "neutralize" natural ligand.

Computer modelling and searching technologies permit identification of compounds, or the improvement of already identified compounds, that can modulate target or pathway
30 gene expression or activity. Having identified such a compound or composition, the active sites or regions are identified.

In the case of compounds affecting receptor molecules, such active sites might typically be ligand binding sites,
35 such as the interaction domains of ligand with receptor itself. The active site can be identified using methods known in the art including, for example, from the amino acid

sequences of peptides, from the nucleotide sequences of nucleic acids, or from study of complexes of the relevant compound or composition with its natural ligand. In the latter case, chemical or X-ray crystallographic methods can
5 be used to find the active site by finding where on the factor the complexed ligand is found.

Next, the three dimensional geometric structure of the active site is determined. This can be done by known methods, including X-ray crystallography, which can determine
10 a complete molecular structure. On the other hand, solid or liquid phase NMR can be used to determine certain intramolecular distances. Any other experimental method of structure determination can be used to obtain partial or complete geometric structures. The geometric structures may
15 be measured with a complexed ligand, natural or artificial, which may increase the accuracy of the active site structure determined.

If an incomplete or insufficiently accurate structure is determined, the methods of computer based numerical modelling
20 can be used to complete the structure or improve its accuracy. Any recognized modelling method may be used, including parameterized models specific to particular biopolymers such as proteins or nucleic acids, molecular dynamics models based on computing molecular motions,
25 statistical mechanics models based on thermal ensembles, or combined models. For most types of models, standard molecular force fields, representing the forces between constituent atoms and groups, are necessary, and can be selected from force fields known in physical chemistry. The
30 incomplete or less accurate experimental structures can serve as constraints on the complete and more accurate structures computed by these modeling methods.

Finally, having determined the structure of the active site, either experimentally, by modeling, or by a
35 combination, candidate modulating compounds can be identified by searching databases containing compounds along with information on their molecular structure. Such a search seeks

compounds having structures that match the determined active site structure and that interact with the groups defining the active site. Such a search can be manual, but is preferably computer assisted. These compounds found from this search
5 are potential target or pathway gene product modulating compounds.

Alternatively, these methods can be used to identify improved modulating compounds from an already known modulating compound or ligand. The composition of the known
10 compound can be modified and the structural effects of modification can be determined using the experimental and computer modelling methods described above applied to the new composition. The altered structure is then compared to the active site structure of the compound to determine if an
15 improved fit or interaction results. In this manner systematic variations in composition, such as by varying side groups, can be quickly evaluated to obtain modified modulating compounds or ligands of improved specificity or activity.

20 Further experimental and computer modeling methods useful to identify modulating compounds based upon identification of the active sites of target or pathway gene or gene products and related transduction and transcription factors will be apparent to those of skill in the art.

25 Examples of molecular modelling systems are the CHARMM and QUANTA programs (Polygen Corporation, Waltham, MA). CHARMM performs the energy minimization and molecular dynamics functions. QUANTA performs the construction, graphic modelling and analysis of molecular structure.
30 QUANTA allows interactive construction, modification, visualization, and analysis of the behavior of molecules with each other.

A number of articles review computer modelling of drugs interactive with specific proteins, such as Rotivinen, et
35 al., 1988, Acta Pharmaceutical Fennica 97:159-166; Ripka, New Scientist 54-57 (June 16, 1988); McKinaly and Rossmann, 1989, Annu. Rev. Pharmacol. Toxicol. 29:111-122; Perry and Davies,

OSAR: Quantitative Structure-Activity Relationships in Drug Design pp. 189-193 (Alan R. Liss, Inc. 1989); Lewis and Dean, 1989 Proc. R. Soc. Lond. 236:125-140 and 1-162; and, with respect to a model receptor for nucleic acid components, 5 Askew, et al., 1989, J. Am. Chem. Soc. 111:1082-1090. Other computer programs that screen and graphically depict chemicals are available from companies such as BioDesign, Inc. (Pasadena, CA.), Allelix, Inc. (Mississauga, Ontario, Canada), and Hypercube, Inc. (Cambridge, Ontario). Although 10 these are primarily designed for application to drugs specific to particular proteins, they can be adapted to design of drugs specific to regions of DNA or RNA, once that region is identified.

Although generally described above with reference to 15 design and generation of compounds which could alter binding, one could also screen libraries of known compounds, including natural products or synthetic chemicals, and biologically active materials, including proteins, for compounds which are inhibitors or activators.

20 Compounds identified via assays such as those described herein can be useful, for example, in elaborating the biological function of the target gene product, and for ameliorating the symptoms of immune disorders. In instances, for example, in which a TH cell subpopulation-related 25 disorder situation results from a lower overall level of target gene expression, target gene product, and/or target gene product activity in a cell or tissue involved in such a disorder, compounds that interact with the target gene product can include ones which accentuate or amplify the 30 activity of the bound target gene protein. Such compounds would bring about an effective increase in the level of target gene activity, thus ameliorating symptoms. In instances whereby mutations within the target gene cause aberrant target gene proteins to be made which have a 35 deleterious effect that leads to a TH cell subpopulation-related disorder, or, alternatively, in instances whereby normal target gene activity is necessary for a TH cell

subpopulation-related disorder to occur, compounds that bind target gene protein can be identified that inhibit the activity of the bound target gene protein. Assays for identifying additional compounds as well as for testing the effectiveness of compounds, identified by, for example, techniques, such as those described in Section 5.8.1-5.8.3, are discussed, below, in Section 5.8.4.

5.8.1. IN VITRO SCREENING ASSAYS FOR COMPOUNDS THAT BIND TO A TARGET GENE PRODUCT

In vitro systems can be designed to identify compounds capable of binding the target gene products of the invention. Compounds identified can be useful, for example, in modulating the activity of wild type and/or mutant target gene products, can be useful in elaborating the biological function of target gene products, can be utilized in screens for identifying compounds that disrupt normal target gene product interactions, or can in themselves disrupt such interactions.

The principle of the assays used to identify compounds that bind to the target gene product involves preparing a reaction mixture of the target gene product and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex which can be removed and/or detected in the reaction mixture. These assays can be conducted in a variety of ways. For example, one method to conduct such an assay would involve anchoring target gene product or the test substance onto a solid phase and detecting target gene product/test compound complexes anchored on the solid phase at the end of the reaction. In one embodiment of such a method, the target gene product can be anchored onto a solid surface, and the test compound, which is not anchored, can be labeled, either directly or indirectly.

In practice, microtiter plates can conveniently be utilized as the solid phase. The anchored component can be immobilized by non-covalent or covalent attachments. Non-

covalent attachment can be accomplished by simply coating the solid surface with a solution of the protein and drying.

Alternatively, an immobilized antibody, preferably a monoclonal antibody, specific for the protein to be

5 immobilized can be used to anchor the protein to the solid surface. The surfaces can be prepared in advance and stored.

In order to conduct the assay, the nonimmobilized component is added to the coated surface containing the anchored component. After the reaction is complete,

10 unreacted components are removed (e.g., by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously nonimmobilized component is
15 pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously nonimmobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the
20 previously nonimmobilized component (the antibody, in turn, can be directly labeled or indirectly labeled with a labeled anti-Ig antibody).

Alternatively, a reaction can be conducted in a liquid phase, the reaction products separated from unreacted

25 components, and complexes detected; e.g., using an immobilized antibody specific for target gene product or the test compound to anchor any complexes formed in solution, and a labeled antibody specific for the other component of the possible complex to detect anchored complexes.

30 Using the 103 gene product as an example, and not by way of limitation, techniques such as those described in this section can be utilized to identify compounds which bind to the 103 gene product. For example, a 103 gene product can be contacted with a compound for a time sufficient to form a 103
35 gene product/compound complex and then such a complex can be detected.

Alternatively, the compound can be contacted with the 103 gene product in a reaction mixture for a time sufficient to form a 103 gene product/compound complex, and then such a complex can be separated from the reaction mixture.

5 Among the 103 gene products which can be utilized for such methods are, for example, rat, murine and human 103 gene products, including, but not limited to the 103 gene products listed in SEQ ID NOS: 39, 41, 43, 45, 47 or 48 (with or without signal peptide sequences) or a naturally occurring
10 variant thereof.

 The term "naturally occurring variant," as used herein refers to an amino acid sequence homologous to the 103 gene product in the same or a different species, such as, for example, an allelic variant of the 103 gene product which
15 maps to the same chromosomal location as the nucleotide sequences encoding the 103 gene products of SEQ ID NOS: 39, 41, 43, 45, 47 or 48, or a location syntenic to such a location. Among the allelic variants which can be utilized herein are allelic variant sequences encoded by a nucleotide
20 sequence that hybridizes under stringent conditions to the complement of a nucleotide sequence encoding the 103 gene products described above (that is SEQ ID NOS: 39, 41, 43, 45, 47 or 48, such as, for example, SEQ ID NOS: 38, 40, 42, 44, 46 or 49.

25

5.8.2. ASSAYS FOR CELLULAR PROTEINS THAT INTERACT WITH THE TARGET GENE PROTEIN

Any method suitable for detecting protein-protein interactions can be employed for identifying novel target
30 protein-cellular or extracellular protein interactions. These methods are outlined in Section 5.2., above, for the identification of pathway genes, and can be utilized herein with respect to the identification of proteins which interact with identified target proteins.

35

5.8.3. ASSAYS FOR COMPOUNDS THAT INTERFERE WITH TARGET GENE PRODUCT/CELLULAR MACROMOLECULE INTERACTION

The target gene products of the invention can, in vivo, interact with one or more cellular or extracellular macromolecules, such as proteins. Such macromolecules can include, but are not limited to, nucleic acid molecules and
5 those proteins identified via methods such as those described, above, in Section 5.8.2. For purposes of this discussion, such cellular and extracellular macromolecules are referred to herein as "binding partners". Compounds that disrupt such interactions can be useful in regulating the
10 activity of the target gene protein, especially mutant target gene proteins. Such compounds can include, but are not limited to molecules such as antibodies, peptides, and the like, as described, for example, in Section 5.8.1. above.

The basic principle of the assay systems used to
15 identify compounds that interfere with the interaction between the target gene product and its cellular or extracellular binding partner or partners involves preparing a reaction mixture containing the target gene product and the binding partner under conditions and for a time sufficient to
20 allow the two to interact and bind, thus forming a complex. In order to test a compound for inhibitory activity, the reaction mixture is prepared in the presence and absence of the test compound. The test compound can be initially included in the reaction mixture, or can be added at a time
25 subsequent to the addition of target gene product and its cellular or extracellular binding partner. Control reaction mixtures are incubated without the test compound or with a placebo. The formation of any complexes between the target gene protein and the cellular or extracellular binding
30 partner is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the compound interferes with the interaction of the target gene protein and the interactive binding partner. Additionally, complex formation
35 within reaction mixtures containing the test compound and normal target gene protein can also be compared to complex formation within reaction mixtures containing the test

compound and a mutant target gene protein. This comparison can be important in those cases wherein it is desirable to identify compounds that disrupt interactions of mutant but not normal target gene proteins.

5 The assay for compounds that interfere with the interaction of the target gene products and binding partners can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring either the target gene product or the binding partner onto a solid phase and
10 detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested. For example,
15 test compounds that interfere with the interaction between the target gene products and the binding partners, e.g., by competition, can be identified by conducting the reaction in the presence of the test substance; i.e., by adding the test substance to the reaction mixture prior to or simultaneously
20 with the target gene protein and interactive cellular or extracellular binding partner. Alternatively, test compounds that disrupt preformed complexes, e.g. compounds with higher binding constants that displace one of the components from the complex, can be tested by adding the test compound to the
25 reaction mixture after complexes have been formed. The various formats are described briefly below.

In a heterogeneous assay system, either the target gene protein or the interactive cellular or extracellular binding partner, is anchored onto a solid surface, while the non-
30 anchored species is labeled, either directly or indirectly. In practice, microtiter plates are conveniently utilized. The anchored species can be immobilized by non-covalent or covalent attachments. Non-covalent attachment can be accomplished simply by coating the solid surface with a
35 solution of the target gene product or binding partner and drying. Alternatively, an immobilized antibody specific for the species to be anchored can be used to anchor the species

to the solid surface. The surfaces can be prepared in advance and stored.

In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (e.g., by washing) and any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, can be directly labeled or indirectly labeled with a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds which inhibit complex formation or which disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, test compounds which inhibit complex or which disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used. In this approach, a preformed complex of the target gene protein and the interactive cellular or extracellular binding partner is prepared in which either the target gene product or its binding partner is labeled, but the signal generated by the label is quenched due to complex formation (see, e.g., U.S. Patent

No. 4,109,496 by Rubenstein which utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal
5 above background. In this way, test substances which disrupt target gene protein/cellular or extracellular binding partner interaction can be identified.

In a particular embodiment, the target gene product can be prepared for immobilization using recombinant DNA
10 techniques described in Section 5.5, above. For example, the target gene coding region can be fused to a glutathione-S-transferase (GST) gene using a fusion vector, such as pGEX-5X-1, in such a manner that its binding activity is maintained in the resulting fusion protein. The interactive
15 cellular or extracellular binding partner can be purified and used to raise a monoclonal antibody, using methods routinely practiced in the art and described above, in Section 5.6. This antibody can be labeled with the radioactive isotope ¹²⁵I, for example, by methods routinely practiced in the art.
20 In a heterogeneous assay, e.g., the GST-target gene fusion protein can be anchored to glutathione-agarose beads. The interactive cellular or extracellular binding partner can then be added in the presence or absence of the test compound in a manner that allows interaction and binding to occur. At
25 the end of the reaction period, unbound material can be washed away, and the labeled monoclonal antibody can be added to the system and allowed to bind to the complexed components. The interaction between the target gene protein and the interactive cellular or extracellular binding partner
30 can be detected by measuring the amount of radioactivity that remains associated with the glutathione-agarose beads. A successful inhibition of the interaction by the test compound will result in a decrease in measured radioactivity.

Alternatively, the GST-target gene fusion protein and
35 the interactive cellular or extracellular binding partner can be mixed together in liquid in the absence of the solid glutathione-agarose beads. The test compound can be added

either during or after the species are allowed to interact. This mixture can then be added to the glutathione-agarose beads and unbound material is washed away. Again the extent of inhibition of the target gene product/binding partner
5 interaction can be detected by adding the labeled antibody and measuring the radioactivity associated with the beads.

In another embodiment of the invention, these same techniques can be employed using peptide fragments that correspond to the binding domains of the target gene product
10 and/or the interactive cellular or extracellular binding partner (in cases where the binding partner is a protein), in place of one or both of the full length proteins. Any number of methods routinely practiced in the art can be used to identify and isolate the binding sites. These methods
15 include, but are not limited to, mutagenesis of the gene encoding one of the proteins and screening for disruption of binding in a co-immunoprecipitation assay. Compensating mutations in the gene encoding the second species in the complex can then be selected. Sequence analysis of the genes
20 encoding the respective proteins will reveal the mutations that correspond to the region of the protein involved in interactive binding. Alternatively, one protein can be anchored to a solid surface using methods described in this Section above, and allowed to interact with and bind to its
25 labeled binding partner, which has been treated with a proteolytic enzyme, such as trypsin. After washing, a short, labeled peptide comprising the binding domain can remain associated with the solid material, which can be isolated and identified by amino acid sequencing. Also, once the gene
30 coding for the cellular or extracellular binding partner is obtained, short gene segments can be engineered to express peptide fragments of the protein, which can then be tested for binding activity and purified or synthesized.

For example, and not by way of limitation, a target gene
35 product can be anchored to a solid material as described, above, in this Section, by making a GST-target gene fusion protein and allowing it to bind to glutathione agarose beads.

The interactive cellular or extracellular binding partner can be labeled with a radioactive isotope, such as ^{35}S , and cleaved with a proteolytic enzyme such as trypsin. Cleavage products can then be added to the anchored GST-target gene fusion protein and allowed to bind. After washing away unbound peptides, labeled bound material, representing the cellular or extracellular binding partner binding domain, can be eluted, purified, and analyzed for amino acid sequence by well known methods. Peptides so identified can be produced synthetically or fused to appropriate facilitative proteins using well known recombinant DNA technology.

5.8.4. ASSAYS FOR AMELIORATION OF IMMUNE DISORDER SYMPTOMS AND/OR THE MODULATION OF TARGET GENE PRODUCT FUNCTION

Any of the binding compounds, including but not limited to, compounds such as those identified in the foregoing assay systems, can be tested for the ability to ameliorate symptoms of immune disorders e.g., TH cell subpopulation-related disorders. Cell-based and animal model-based assays for the identification of compounds exhibiting such an ability to ameliorate immune disorder symptoms are described below. Further, cell-based assays for the identification of compounds which modulate target gene product function, in instances where the target gene product is a receptor having a seven transmembrane domain sequence, such as, for example, that of the 10 gene product, are described, below, in Section 5.8.4.1.

First, cell-based systems such as those described, above, in Section 5.7.2, can be used to identify compounds which can act to ameliorate TH cell subpopulation-related disorder symptoms. For example, such cell systems can be exposed to a compound, suspected of exhibiting an ability to ameliorate the disorder symptoms, at a sufficient concentration and for a time sufficient to elicit such an amelioration in the exposed cells. After exposure, the cells are examined to determine whether one or more of the TH cell subpopulation-related disorder-like cellular phenotypes has

been altered to resemble a phenotype more likely to produce a lower incidence or severity of disorder symptoms. Additional cell-based assays are discussed, below, in Section 5.8.4.1.

Taking the TH cell subpopulation-related disorder
5 asthma, which is, specifically, a TH2-like-related disorder, any TH2 or TH2-like cell system can be utilized. Upon exposure to such cell systems, compounds can be assayed for their ability to modulate the TH2-like phenotype of such cells, such that the cells exhibit loss of a TH2-like
10 phenotype. Compounds with such TH2 modulatory capability represent ones which can potentially exhibit the ability to ameliorate asthma-related symptoms in vivo. The Example presented in Section 12, below, describes the successful utilization of a 103 gene product/Ig fusion protein, as well
15 as the successful use of a monoclonal antibody directed against the extracellular domain of the 103 gene product to ameliorate symptoms of asthma in an accepted animal model of asthma.

In addition, animal-based systems, such as those
20 described, above, in Section 5.7.1, can be used to identify compounds capable of ameliorating TH cell subpopulation-related disorder-like symptoms. Such animal models can be used as test substrates for the identification of drugs, pharmaceuticals, therapies, and interventions which can be
25 effective in treating such disorders. For example, animal models can be exposed to a compound, suspected of exhibiting an ability to ameliorate TH cell subpopulation-related disorder symptoms, at a sufficient concentration and for a time sufficient to elicit such an amelioration of the
30 symptoms in the exposed animals. The response of the animals to the exposure, and thus the efficacy of the compound in question, can be monitored by assessing the reversal of disorders associated with TH cell subpopulation-related disorders of interest. With regard to intervention, any
35 treatments which reverse any aspect of TH cell subpopulation-related disorder-like symptoms should be considered as candidates for corresponding human TH cell subpopulation-

related disorder therapeutic intervention. Dosages of test agents can be determined by deriving dose-response curves, as discussed in Section 5.11, below.

Gene expression patterns can be utilized in conjunction with either cell-based or animal-based systems, to assess the ability of a compound to ameliorate TH cell subpopulation-related disorder-like symptoms. For example, the expression pattern of one or more fingerprint genes can form part of a fingerprint profile which can be then be used in such an assessment. Fingerprint profiles are described, below, in Section 5.12. Fingerprint profiles can be characterized for known states, either TH cell subpopulation-related disorder states, or normal TH cell differentiative states, within the cell- and/or animal-based model systems.

15

5.9. METHODS FOR THE IDENTIFICATION OF COMPOUNDS WHICH MODULATE TARGET GENE PRODUCT FUNCTION

In this Section, methods are described for the identification of compounds which act as either agonists or antagonists of receptor target gene products. The 10 gene product (FIG. 9; SEQ ID NO:9) is an example of a seven transmembrane domain target gene product. For ease of explanation, and not by way of limitation, therefore, the 10 gene product will be used to illustrate the methods described in this Section.

25

The compounds tested may be, for example, compounds such as those identified via the assays described, above, in Sections 5.8.1 to 5.8.3. Such compounds may include, but are not limited to peptides such as, for example, soluble peptides, including, but not limited to, Ig-tailed fusion peptides, comprising extracellular portions of target gene product transmembrane receptors, and members of random peptide libraries (see, e.g., Lam, K.S. et al., 1991, Nature 354:82-84; Houghten, R. et al., 1991, Nature 354:84-86) made of D-and/or L-configuration amino acids, phosphopeptides (including but not limited to members of random or partially degenerate, directed phosphopeptide libraries; see, e.g.,

35

Songyang, Z. et al., 1993, Cell 72:767-778), antibodies (including, but not limited to polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and FAb, F(ab')₂ and FAb expression library
5 fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules.

The assays described herein are functional assays which identify compounds that affect the receptor target gene's activity by affecting the level of intracellular calcium
10 release within cells expressing such seven transmembrane domain receptor target protein (e.g., the 10 gene product). Intracellular calcium release is measured because such seven transmembrane domain receptors tend to be G protein-coupled receptors and because activation of these receptors leads to
15 a G protein-mediated intracellular calcium release. Modulation (i.e., agonization or antagonization) of the receptor target gene product function, then, would result in a difference in intracellular calcium levels.

The assays comprise contacting a seven transmembrane
20 domain receptor target gene-expressing cell with a test compound and measuring the level of intracellular calcium. Those compounds which produce an intracellular calcium profile which differs from that which the cell would exhibit in the absence of the compound represent either agonists or
25 antagonists. An agonist compound would cause an increase in intracellular calcium levels relative to control cells while an antagonist would result in a decrease in intracellular calcium levels relative to control cells.

While any cell expressing a seven transmembrane receptor
30 target gene product may be used herein, it is preferred that cells be used whose intracellular calcium levels may readily be measured. Xenopus oocytes, due to their large size, are among such preferred cells because they can easily be injected with intracellular calcium reporter compounds.
35 Additionally, myeloma cells may be utilized. Such reporter compounds include, but are not limited to, calcium-binding agents such as the well known FURA-2 and INDO-2. FURA-

2/calcium complexes and INDO-2/calcium complexes fluoresce, making possible the measurement of differences in intracellular calcium levels.

For the purposes of the assays described herein, the
5 Xenopus oocytes should be transfected with nucleotide sequences encoding the target protein of interest (e.g., the 10 gene product). The cells can be transfected and express the sequence of interest via techniques which are well known to those of skill in the art and which may include, for
10 example, techniques such as those described, above, in Section 5.5. Xenopus oocytes can be injected with RNA encoding the target gene product of interest such that the injected oocytes will express the gene product.

The assays described in this Section may, first, be used
15 to identify compounds which act as agonists of the target gene product of interest, e.g., the 10 gene product.

"Agonist", as used herein, refers to a compound which modulates target gene product activity by increasing the target gene product's activity, as evaluated by the
20 compound's ability to bring about an increase in calcium influx, leading to an increase intracellular calcium levels. Among such agonists can be, for example, the natural ligand for the receptor target gene product, e.g., the natural ligand for the 10 gene product.

25 Agonists identified via such assays may act as useful therapeutic agents for the amelioration of a wide range of T cell-related disorders, including, for example, TH cell subpopulation-related disorders, in instances whereby such disorders are caused by a reduced or absent level of target
30 gene product activity. Any of the agonist compounds identified herein can be used, for example, as part of the treatment methods described in Section 5.9.2, below.

Further, such agonists can be used to identify antagonists of the receptor target gene product of interest, e.g., as
35 described, below.

"Antagonist", as used herein, refers to a compound which modulates target gene product activity by decreasing the

target gene product's activity, as evaluated by the compound's ability to bring about a decrease in calcium influx. Antagonists identified via such assays may act as useful therapeutic agents for the amelioration of a wide
5 range of T cell-related disorders, including, for example, TH cell subpopulation-related disorders, in instances whereby the disorder is caused by an increased or inappropriate level of target gene product activity.

An antagonist screen may be performed utilizing target
10 gene product-expressing cells as described, above, and which include, but are not limited to, such cells as 10 gene-expressing cells, for example, 10 gene-expressing Xenopus oocytes. In those instances whereby the T cell-related disorder is caused by a mutant target gene product, the cells
15 utilized in the antagonist assay can be cells which express the mutant receptor target gene product involved in causing the T cell-related disorder.

To conduct an antagonist screen, a target gene-expressing cell is contacted with 1) an agonist of the target
20 gene product and 2) a test compound for a given period of time. The level of intracellular calcium is then measured in the cells and in cells which have been contacted with agonist alone. A test compound is considered to be an antagonist if the level of intracellular calcium release in the presence of
25 the test compound is lower than the level of intracellular calcium release in the absence of the test compound.

Any of the antagonist compounds identified herein can be used, for example, as part of the treatment methods described, below, in Section 5.9.1.

30 Among the potential antagonist compounds of the seven transmembrane domain receptor target gene products described herein are peptides which contain one or more of the receptor target gene product's extracellular domains, preferably those domains are domains which are responsible for ligand-binding
35 such that the peptides act to compete with the endogenous receptor for ligand. In the case of the 10 gene product, for example, such extracellular domains include from

approximately 10 gene product amino acid residue 1 to 19, amino acid residue 74 to 87, amino acid residue 153-187 and amino acid residue 254 to 272. Such extracellular domain antagonist compounds may comprise soluble Ig-tailed fusion
5 proteins which may be produced by utilizing techniques such as those described, above, in Section 5.5. Additionally, antibodies directed against the extracellular portion of the 10 gene product may reduce 10 gene product function by, for example, blocking ligand binding.

10

5.10. COMPOUNDS AND METHODS FOR TREATMENT OF IMMUNE DISORDERS AND FOR MODULATION OF TH CELL RESPONSIVENESS

Described below are methods and compositions which can be used to ameliorate immune disorder symptoms via, for
15 example, a modulation of the TH cell subpopulation of interest. Such modulation can be of a positive or negative nature, depending on the specific situation involved, but each modulatory event yields a net result in which symptoms of the immune disorder are ameliorated. Further, described
20 below are methods for the modulation of TH cell responsiveness to antigen.

"Negative modulation", as used herein, refers to a reduction in the level and/or activity of target gene product relative to the level and/or activity of the target gene
25 product in the absence of the modulatory treatment. Alternatively, the term, as used herein, refers to a depletion of the T cell subpopulation (e.g., via a reduction in the number of cells belonging to the TH cell subpopulation) relative to the number present in the absence
30 of the modulatory treatment. "Depletion," as used herein, is as defined, above, in Section 3.

"Positive modulation", as used herein, refers to an increase in the level and/or activity of target gene product relative to the level and/or activity of the gene product in
35 the absence of the modulatory treatment. Alternatively, the term, as used herein, refers to a stimulation of the T cell subpopulation (e.g., via an increase in the number of cells

belonging to the TH cell subpopulation), relative to the number present in the absence of the modulatory treatment. "Stimulation," as used herein, is as defined, above, in Section 3.

5 It is possible that a TH cell subpopulation-related disorder or other immune disorder, can occur as a result of normal target gene activity during the course of, for example, exposure to a certain antigen which elicits an immune response that leads to the development of the
10 disorder. For example, the TH2-like-related disorders, asthma and allergy, are likely candidates of disorders having such a mechanism. Additionally, a disorder can be brought about, at least in part, by an abnormally high level of target gene product, or by the presence of a target gene
15 product exhibiting an abnormal activity. As such, a technique which elicits a negative modulatory effect, i.e., brings about a reduction in the level and/or activity of target gene product, or alternatively, brings about a depletion of the TH cell subpopulation (e.g., via a physical
20 reduction in the number of cells belonging to the TH cell subpopulation), would effect an amelioration of TH cell subpopulation-related disorder symptoms in either of the above scenarios.

Negative modulatory techniques for the reduction of
25 target gene expression levels or target gene product activity levels, (either normal or abnormal), and for the reduction in the number of specific TH cell subpopulation cells are discussed in Section 5.9.1, below.

Alternatively, it is possible that a TH cell
30 subpopulation-related disorder or other immune disorders can be brought about, at least in part, by the absence or reduction of the level of target gene expression, a reduction in the level of a target gene product's activity, or a reduction in the overall number of cells belonging to a
35 specific TH cell subpopulation. As such, a technique which elicits a positive modulatory effect, i.e., brings about an increase in the level of target gene expression and/or the

activity of such gene products, or, alternatively, a stimulation of the TH cell subpopulation (e.g., via a physical increase in the number of cells belonging to a TH cell subpopulation), would effect an amelioration of immune disorder symptoms.

For example, a reduction in the overall number of TH1-like cells relative to TH2-like cells within a HIV-infected individual can correlate with the progression to AIDS (Clerci, M. et al., 1993, J. Clin. Invest. 91:759; Clerci, M. et al., 1993, Science 262:1721; Maggi, E. et al., 1994, Science 265:244). A treatment capable of increasing the number of TH1-like cells relative to TH2-like cells within an HIV-infected individual may, therefore, serve to prevent or slow the progression to disease.

Positive modulatory techniques for increasing target gene expression levels or target gene product activity levels, and for increasing the level of specific TH cell subpopulation cells are discussed, below, in Section 5.9.2.

Among the immune disorders whose symptoms can be ameliorated are TH1 or TH1-like related immune disorders and TH2 or TH2-like related immune disorders. Examples of TH1 or TH1-like related disorders include chronic inflammatory diseases and disorders, such as Crohn's disease, reactive arthritis, including Lyme disease, insulin-dependent diabetes, organ-specific autoimmunity, including multiple sclerosis, Hashimoto's thyroiditis and Grave's disease, contact dermatitis, psoriasis, graft rejection, graft versus host disease and sarcoidosis. Examples of TH2 or TH2-like related disorders include atopic conditions, such as asthma and allergy, including allergic rhinitis, gastrointestinal allergies, including food allergies, eosinophilia, conjunctivitis, glomerular nephritis, certain pathogen susceptibilities such as helminthic (e.g., leishmaniasis) and certain viral infections, including HIV, and bacterial infections, including tuberculosis and lepromatous leprosy.

The methods described herein can additionally be utilized to modulate the level of responsiveness, for

example, responsiveness to antigen, of a TH cell subpopulation. Such methods are important in that many immune disorders involve inappropriate rather than insufficient immune responses. For example, disorders such as atopic, IgE-mediated allergic conditions, including asthma, pathogen susceptibilities and chronic inflammatory disease, involve strong but counterproductive TH2-mediated immune responses. Further, inappropriate TH1-mediated immune responses to self-antigens is central to the development of such disorders as multiple sclerosis, psoriasis, insulin dependent diabetes, Hashimoto's thyroiditis and Crohn's disease.

Methods for modulating TH cell responsiveness can comprise, for example, contacting a compound to a TH cell so that the responsiveness of the T helper cell is modulated relative to the responsiveness of the T helper cell in the absence of the compound. The modulation can increase or decrease the responsiveness of the TH cell. Any of the techniques described, below, in Sections 5.9.1-5.9.3.2 can be utilized to effect an appropriate modulation of TH cell responsiveness.

Still further, the methods and techniques described herein can also be used for treating, ameliorating, or modulating symptoms associated with mast cell-related processes or disorders or certain ischemia disorders or injuries. For example, techniques which increase the expression or activity of certain gene products of the invention whose activity is involved in the repair of ischemic injury or damage, particularly techniques which increase the expression or activity of the 200 gene product of this invention, can be used to treat tissue or organ damage produced by an ischemic disorder or injury. Alternatively, techniques or methods which inhibit the expression or activity of target gene products of the invention can be used to block or inhibit the repair of ischemic tissue or organs. Such techniques are useful, for example, to treat ischemic or infarcted tissue, such as a

cancerous tumor, to increase damage or injury to such tissue, e.g., during treatment such as chemotherapy.

Among the ischemic disorders or injuries whose symptoms can be ameliorated are ischemic renal disease and myocardial ischemia, such as angina pectoris, as well as ischemic injuries to other tissues including, but by no means limited to, the brain (as in a stroke), spleen, intestine, lung, and testes. Such techniques can also be used to treat, or to enhance ischemic injuries to tumors, including tumors of the ovary or uterus.

The methods described herein can additionally be used to treat or prevent ischemic damage or injury to transplanted organs, such as transplanted kidneys, lungs, hearts, livers, and pancreas, or grafts, such as skin grafts. Such techniques are important in that some ischemic damage to transplanted organs typically occurs during transplant from donor to host, when oxygen perfusion to tissue of the transplanted organ is severely reduced.

Among the genes and gene products identified and present in the present invention, the 200 gene is demonstrated in Section 13, herein, to play a critical role in the resolution (i.e., the repair) of injury following ischemia reperfusion. The 200 gene and its products can, therefore, be utilized in the treatment of ischemic disorders and injuries. For example, a gene 200 product, or functional portions thereof, can be utilized either directly or indirectly to stimulate or increase the repair of injury to tissue or organs resulting from an ischemic injury or disorder.

30

5.10.1. NEGATIVE MODULATORY TECHNIQUES

As discussed, above, successful treatment of certain immune disorders can be brought about by techniques which serve to inhibit the expression or activity of target gene products, or which, alternatively, serve to reduce the overall number of cells belonging to a specific TH cell subpopulation. Further, as also described above, techniques

which serve to inhibit the expression or activity of target gene products of the present invention, including the 200 gene product, can be used to inhibit the repair or recovery of certain ischemic tissue.

5 For example, compounds such as those identified through assays described, above, in Section 5.8, which exhibit negative modulatory activity, can be used in accordance with the invention to ameliorate certain TH cell subpopulation-related disorder symptoms, or, alternatively, to inhibit the
10 repair or recovery of ischemic tissue. As discussed in Section 5.8, above, such molecules can include, but are not limited to peptides (such as, for example, peptides representing soluble extracellular portions of target gene product transmembrane receptors), phosphopeptides, small
15 organic or inorganic molecules, or antibodies (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and FAb, F(ab')₂, and FAb expression library fragments, and epitope-binding fragments thereof). In one embodiment, for example, antibodies
20 directed against a 103 gene product, preferably an extracellular or extracellular portion of a 103 gene product, can be utilized. Techniques for the determination of effective doses and administration of such compounds are described, below, in Section 5.11.

25 Further, antisense and ribozyme molecules which inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity. Still further, triple helix molecules can be
30 utilized in reducing the level of target gene activity. Such techniques are described, below.

Additionally, techniques for the depletion of specific TH cell subpopulations are discussed, below, in Section 5.10.3.2. Such techniques can take advantage of, for
35 example, novel cell surface markers which are specific to the TH cell subpopulation to be depleted, and can include in vivo or in vitro targeted destruction, or, alternatively,

selective purification away, of the TH cell subpopulation of interest.

Among the TH cell subpopulation-related sequences identified by the methods described by the present invention is a gene designated herein as the 103 gene, as discussed in the Example presented in Section 7, below. The 103 gene is demonstrated herein to represent a TH2-specific gene in that 103 gene expression is found to be absent TH1 cells as well as all other tissues tested. Further, at least one of the proteins produced by the 103 gene is a transmembrane protein.

The 103 gene and its products can, therefore, be utilized in the treatment of TH2 cell subpopulation-related disorders. For example, a 103 gene product or portions thereof can be utilized, either directly or indirectly, to ameliorate conditions involving inappropriate IgE immune responses, including, but not limited to the symptoms which accompany atopic conditions such as allergy and/or asthma. IgE-type antibodies are produced by stimulated B cells which require, at least in part, IL-4 produced by the TH2 cell subpopulation. Therefore, any treatment, including, for example, the use of a gene 103 product or portion thereof, which reduces the effective concentration of secreted IL-4, e.g., by reducing the number or activity of TH2 cells, can bring about a reduction in the level of circulating IgE, leading, in turn, to the amelioration of the conditions stemming from an inappropriate IgE immune response.

There exist a variety of ways in which the TH2 specific 103 gene products can be used to effect such a reduction in the activity and/or effective concentration of TH2 cells.

For example, natural ligands, derivatives of natural ligands and antibodies which bind to the 103 gene product can be utilized to reduce the number of TH2 cells present by either physically separating such cells away from other cells in a population, thereby deleting the TH2 cell subpopulation, or, alternatively, by targeting the specific destruction of TH2 cells. Such techniques are discussed, below, in Section

5.9.3. Further, such compounds can be used to inhibit the proliferation of TH2 cells.

Additionally, compounds such as 103 gene sequences or gene products can be utilized to reduce the level of TH2 cell activity, cause a reduction in IL-4 production, and, ultimately, bring about the amelioration of IgE related disorders.

For example, compounds can be administered which compete with endogenous ligand for the 103 gene product. The resulting reduction in the amount of ligand-bound 103 gene transmembrane protein will modulate TH2 cellular activity. Compounds which can be particularly useful for this purpose include, for example, soluble proteins or peptides, such as peptides comprising the extracellular domain, or portions and/or analogs thereof, of the gene 103 product, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins. (For a discussion of the production of Ig-tailed fusion proteins see, for example, U.S. Patent No. 5, 116,964.)

Production of a 103 gene product/Ig fusion is described in Section 10, below. Further, use of a 103 gene product/Ig fusion to successfully ameliorate symptoms in an accepted animal model for asthma is described in Section 12, below.

The novel 200 gene, which encodes a receptor target gene product that is a member of the Ig superfamily, exhibits a TH1-specific pattern of gene expression. The 200 gene, especially the human 200 gene, and its products can, therefore, be utilized in the treatment of TH1 cell subpopulation-related disorders such as, for example, chronic inflammatory diseases, psoriasis, graft rejection and graft versus host disease.

The treatment of such disorder may require a reduction in the activity and/or effective concentration of the TH1 cell subpopulation involved in the disorder of interest. As such, a number of methods exist whereby the TH1 specific 200 gene products can be used to effect such a reduction in the activity and/or effective concentration of TH1 cells.

For example, natural ligands, derivatives of natural ligands and antibodies which bind to the 200 gene product can be utilized to reduce the number of TH1 cells present by either physically separating such cells away from other cells in a population, thereby deleting the TH1 cell subpopulation, or, alternatively, by targeting the specific destruction of TH1 cells. Such techniques are discussed, below, in Section 5.10.3. Further, such compounds can be used to inhibit the proliferation of TH1 cells.

10 Additionally, compounds can be administered which compete with endogenous ligand for the 200 gene product. Such compounds would bind to and "neutralize" circulating ligand. The resulting reduction in the amount of ligand-bound 200 gene transmembrane protein will modulate TH1
15 cellular activity. Further, reduction in the amount of ligand bound 200 gene transmembrane protein will also inhibit the resolution and repair of ischemic tissue.

Compounds which can be particularly useful for this purpose include, for example, soluble proteins or peptides,
20 such as peptides comprising the extracellular domain, or portions and/or analogs thereof, of the gene 200 product, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins or antibodies. (For a discussion of the production of Ig-tailed fusion proteins see, for example,
25 U.S. Patent No. 5, 116,964.)

To this end, peptides corresponding to the ECD of the 200 gene product, soluble deletion mutants of 200 gene product, or either of these 200 gene product domains or mutants fused to another polypeptide (e.g., an IgFc
30 polypeptide) can be utilized. Alternatively, anti-idiotypic antibodies or Fab fragments of antiidiotypic antibodies that mimic the 200 gene product ECD and neutralize 200 gene product ligand can be used. Such 200 gene product peptides, proteins, fusion proteins, anti-idiotypic antibodies or Fabs
35 are administered to a subject in amounts sufficient to neutralize the gene product and thereby effectuate an

amelioration of a T cell subpopulation-related disorder, or an inhibition of repair of ischemic tissues.

200 gene product peptides corresponding to the ECD having the amino acid sequence shown in FIG. 17 (SEQ ID NO:10) from about amino acid residue number 21 to about 192 can be used. Human 200 gene product peptides corresponding to the ECD having the amino acid sequence shown in FIG. 24 (SEQ ID NO:24) from approximately amino acid residue number 21 to about 200. Mutants in which all or part of the hydrophobic anchor sequence (e.g., about amino acid residue number 193 to 214 in FIG. 17, or about 201 to about 224 in FIG. 24) is deleted could also be used. Fusion of these peptides to an IgFc polypeptide should not only increase the stability of the preparation, but will increase the half-life and activity of the fusion protein in vivo. The Fc region of the Ig portion of the fusion protein may be further modified to reduce immunoglobulin effector function. For example, nucleotide sequences encoding the fusion protein may be modified to encode fusion proteins which replace cysteine residues in the hinge region with serine residues and/or amino acids within the CH2 domain believed to be required for IgC binding to FC receptors and complement activation.

In an alternative embodiment for neutralizing circulating 200 gene product ligand, cells that are genetically engineered to express such soluble or secreted forms of 200 gene product may be administered to a patient, whereupon they will serve as "bioreactors" *in vivo* to provide a continuous supply of the 200 gene product ligand neutralizing protein. Such cells may be obtained from the patient or an MHC compatible donor and can include, but are not limited to fibroblasts, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence for the 200 gene product peptide, or 200 gene product fusion proteins (discussed above) into the cells, e.g., by transduction (using viral vectors, and preferably vectors that integrate

the transgene into the cell genome) or transfection procedures, including but not limited to the use of plasmids, cosmids, YACs, electroporation, liposomes, etc. The 200 gene product coding sequence can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression and secretion of the 200 gene peptide or fusion protein. The engineered cells which express and secrete the desired 200 gene product can be introduced into the patient systemically, e.g., in the circulation, or intrapertioneally. Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

It is to be understood that, while such approaches and techniques are described, for sake of clarity, using the 200 gene product as an example, they may be applied to any of the target and/or pathway gene products having such receptor-type structures.

The 10 gene product is identified herein as a receptor target gene product having a seven transmembrane domain sequence motif. Further, the 10 gene is shown to exhibit a TH inducible pattern of expression, meaning that 10 gene expression increases in both TH1 and TH2 cell subpopulations in response to stimulation and can important to T cell responses in general. The 10 gene and its products can,

therefore, be utilized in the treatment of a wide T cell-related disorders. Techniques such as those described, above, for the 103 and the 200 genes and gene products can also be utilized for the amelioration of disorders in which 5 10 gene expression is involved.

Modulatory Antisense, Ribozyme,
and Triple Helix Approaches:

Among the compounds which can exhibit the ability to
10 ameliorate TH cell subpopulation-related disorder symptoms are antisense, ribozyme, and triple helix molecules. Such molecules can be designed to reduce or inhibit either wild type, or if appropriate, mutant target gene activity. Techniques for the production and use of such molecules are
15 well known to those of skill in the art.

Antisense approaches involve the design of oligonucleotides (either DNA or RNA) that are complementary to target or pathway gene mRNA. The antisense oligonucleotides will bind to the complementary target or
20 pathway gene mRNA transcripts and prevent translation. Absolute complementarity, although preferred, is not required. A sequence "complementary" to a portion of an RNA, as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming
25 a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the
30 longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized
35 complex.

Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and

including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have recently been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., 1994, Nature 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'- non-translated, non-coding regions of target or pathway genes, as shown, for example, in Fig. 9, 17, 22, 23 and 24, could be used in an antisense approach to inhibit translation of endogenous target or pathway gene mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5'-, 3'- or coding region of target or pathway gene mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

Regardless of the choice of target sequence, it is preferred that *in vitro* studies are first performed to quantitate the ability of the antisense oligonucleotide to inhibit gene expression. It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligonucleotides. It is also preferred that these studies compare levels of the target RNA or protein with that of an internal control RNA or protein. Additionally, it is envisioned that results obtained using the antisense oligonucleotide are compared with those obtained using a control oligonucleotide. It is preferred that the control oligonucleotide is of approximately the same length as the test oligonucleotide and that the nucleotide sequence of the oligonucleotide differs from the antisense sequence no more

than is necessary to prevent specific hybridization to the target sequence.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-
5 stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host
10 cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain
15 barrier (see, e.g., PCT Publication No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., 1988, BioTechniques 6:958-976) or intercalating agents. (See, e.g., Zon, 1988, Pharm. Res. 5:539-549). To this end, the oligonucleotide may be
20 conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group
25 including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-
30 galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxycarboxymethyl-2-thiouracil,
35 beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil,

queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) 5 uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

10 In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an 15 alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an α -anomeric oligonucleotide. An α -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, 20 the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

25 Oligonucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the 30 method of Stein et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

The antisense molecules should be delivered to cells 35 which express the target or pathway gene *in vivo*. A number of methods have been developed for delivering antisense DNA or RNA to cells; e.g., antisense molecules can be injected

directly into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

However, it is often difficult to achieve intracellular concentrations of the antisense sufficient to suppress translation of endogenous mRNAs. Therefore a preferred approach utilizes a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong pol III or pol II promoter. The use of such a construct to transfect target cells in the patient will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous target or pathway gene transcripts and thereby prevent translation of the target or pathway gene mRNA. For example, a vector can be introduced in vivo such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42), etc. Any type of plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant DNA construct which can be introduced

directly into the tissue site. Alternatively, viral vectors can be used which selectively infect the desired tissue.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA (For a review see, 5 for example Rossi, J., 1994, Current Biology 4:469-471). The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage. The composition of ribozyme molecules must include one or more 10 sequences complementary to the target gene mRNA, and must include the well known catalytic sequence responsible for mRNA cleavage. For this sequence, see U.S. Pat. No. 5,093,246, which is incorporated by reference herein in its entirety. As such, within the scope of the invention are 15 engineered hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of RNA sequences encoding target gene proteins. Ribozyme molecules designed to catalytically cleave target or pathway gene mRNA transcripts can also be used to prevent 20 translation of target or pathway gene mRNA and expression of target or pathway gene. (See, e.g., PCT International Publication WO90/11364, published October 4, 1990; Sarver et al., 1990, Science 247:1222-1225). While ribozymes that cleave mRNA at site specific recognition sequences can be 25 used to destroy target or pathway gene mRNAs, the use of hammerhead ribozymes is preferred.

Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target 30 mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, 1988, Nature, 334:585-591. Preferably the ribozyme is engineered so that the cleavage recognition site 35 is located near the 5' end of the target or pathway gene mRNA; i.e., to increase efficiency and minimize the

intracellular accumulation of non-functional mRNA transcripts.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as
5 the one which occurs naturally in *Tetrahymena Thermophila* (known as the IVS, or L-19 IVS RNA) and which has been extensively described by Thomas Cech and collaborators (Zaug, et al., 1984, *Science*, 224:574-578; Zaug and Cech, 1986, *Science*, 231:470-475; Zaug, et al., 1986, *Nature*, 324:429-
10 433; published International patent application No. WO 88/04300 by University Patents Inc.; Been and Cech, 1986, *Cell*, 47:207-216). The Cech-type ribozymes have an eight base pair active site which hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place.
15 The invention encompasses those Cech-type ribozymes which target eight base-pair active site sequences that are present in target or pathway gene.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (e.g. for improved
20 stability, targeting, etc.) and should be delivered to cells which express the target or pathway gene *in vivo*. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected
25 cells will produce sufficient quantities of the ribozyme to destroy endogenous target or pathway gene messages and inhibit translation. Because ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

30 In instances wherein the antisense, ribozyme, and/or triple helix molecules described herein are utilized to inhibit mutant gene expression, it is possible that the technique can also efficiently reduce or inhibit the transcription (triple helix) and/or translation (antisense,
35 ribozyme) of mRNA produced by normal target gene alleles that the possibility can arise wherein the concentration of normal target gene product present can be lower than is necessary

for a normal phenotype. In such cases, to ensure that substantially normal levels of target gene activity are maintained, therefore, nucleic acid molecules that encode and express target gene polypeptides exhibiting normal target gene activity can be introduced into cells via gene therapy methods such as those described, below, in Section 5.9.2 that do not contain sequences susceptible to whatever antisense, ribozyme, or triple helix treatments are being utilized. Alternatively, in instances whereby the target gene encodes an extracellular protein, it can be preferable to coadminister normal target gene protein in order to maintain the requisite level of target gene activity.

Anti-sense RNA and DNA, ribozyme, and triple helix molecules of the invention can be prepared by any method known in the art for the synthesis of DNA and RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules can be generated by in vitro and in vivo transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences can be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

Various well-known modifications to the DNA molecules can be introduced as a means of increasing intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences of ribo- or deoxy- nucleotides to the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the oligodeoxyribonucleotide backbone.

Endogenous target and/or pathway gene expression can also be reduced by inactivating or "knocking out" the target

and/or pathway gene or its promoter using targeted homologous recombination. (E.g., see Smithies et al., 1985, Nature 317:230-234; Thomas & Capecchi, 1987, Cell 51:503-512; Thompson et al., 1989 Cell 5:313-321; each of which is
5 incorporated by reference herein in its entirety). For example, a mutant, non-functional target and/or pathway gene (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous target and/or pathway gene (either the coding regions or regulatory regions of the
10 target and/or pathway gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express target and/or pathway gene *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the
15 target and/or pathway gene. Such approaches are particularly suited in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive target and/or pathway gene (e.g., see Thomas & Capecchi 1987 and Thompson 1989, supra). Such
20 techniques can also be utilized to generate T cell subpopulation-related disorder animal models. It should be noted that this approach can be adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site in vivo using
25 appropriate viral vectors, e.g., herpes virus vectors.

Alternatively, endogenous target and/or pathway gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the target and/or pathway gene (i.e., the target and/or pathway
30 gene promoter and/or enhancers) to form triple helical structures that prevent transcription of the target or pathway gene in target cells in the body. (See generally, Helene, C. 1991, Anticancer Drug Des., 6(6):569-84; Helene, C., et al., 1992, Ann, N.Y. Accad. Sci., 660:27-36; and
35 Maher, L.J., 1992, Bioassays 14(12):807-15). In yet another embodiment of the invention, the activity of target and/or pathway gene can be reduced using a "dominant negative"

approach. To this end, constructs which encode defective target and/or pathway gene products can be used in gene therapy approaches to diminish the activity of the target and/or pathway gene product in appropriate target cells.

5

5.10.2. POSITIVE MODULATORY TECHNIQUES

As discussed above, successful treatment of certain immune disorders can be brought about by techniques which serve to increase the level of target gene expression or to
10 increase the activity of target gene product, or which, or alternatively, serve to effectively increase the overall number of cells belonging to a specific TH cell subpopulation. As also discussed above, techniques which serve to increase the level of target gene expression or to
15 increase the activity of target gene product can serve to enhance the repair or resolution of ischemia reperfusion injury to an organ or tissue. Thus, such techniques can also be used to successfully treat ischemic disorders or injuries.

For example, compounds such as those identified through
20 assays described, above, in Section 5.8, which exhibit positive modulatory activity can be used in accordance with the invention to ameliorate certain TH cell subpopulation-related disorder symptoms, or to treat ischemic disorders and injuries. As discussed in Section 5.8, above, such molecules
25 can include, but are not limited to proteins or protein fragments of the target gene product, including fragments corresponding to one or more functional domains of the target gene product (e.g., an extracellular domain, a transmembrane domain, or a cytosolic domain) or portions thereof. Such
30 molecules can also include peptides representing soluble extracellular portions of target gene product transmembrane proteins, phosphopeptides, small organic or inorganic molecules, or antibodies (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single
35 chain antibodies, and FAb, F(ab')₂ and FAb expression library fragments, and epitope-binding fragments thereof).

For example, a compound, such as a target gene protein, can, at a level sufficient to ameliorate immune disorder symptoms, be administered to a patient exhibiting such symptoms. Such compounds can also be administered to a
5 patient having an ischemic disorder or injury at a level sufficient to ameliorate the symptoms of the ischemic disorder or injury. Any of the techniques discussed, below, in Section 5.11, can be utilized for such administration. One of skill in the art will readily know how to determine
10 the concentration of effective, non-toxic doses of the compound, utilizing techniques such as those described, below, in Section 5.11.1.

In another embodiment, fragments or peptides representing a functional domain of a target gene product are
15 administered to an individual at sufficient dosages and such that the fragments or peptides may enhance the target gene product's activity in the individual, e.g., by mimicking the function of the target gene product *in vivo*. For example, in one preferred embodiment, fragments or peptides representing
20 a functional domain of the 200 gene product are administered to a patient which enhance and/or mimic the activity of the endogenous 200 gene product in that patient. Such 200 gene fragments may therefore be used, e.g., to stimulate or increase the repair of injury to tissue or organs resulting
25 from an ischemic injury or disorder.

The proteins and peptides which may be used in such methods include synthetic (e.g., recombinant or chemically synthesized) proteins and peptides, as well as naturally occurring proteins and peptides. The proteins and peptides
30 may have both naturally occurring and/or non-naturally occurring amino acid residues (e.g., D-amino acid residues) and/or one or more non-peptide bonds (e.g., imino, ester, hydrazide, semicarbazide, and azo bonds). The proteins or peptides may also contain additional chemical groups (e.g.,
35 functional groups) present at the amino and/or carboxy termini, such that, for example, the stability, bioavailability, and/or inhibitory activity of the peptide is

enhanced. Exemplary functional groups include hydrophobic groups (e.g., carbobenzoxyl, dansyl, and t-butyloxycarbonyl groups) an acetyl group, a 9-fluorenylmethoxy-carbonyl group, and macromolecular carrier groups (e.g., lipid-fatty acid
5 conjugates, polyethylene glycol, or carbohydrates) including peptide groups. Suitable dosages and formulations for administration of such peptides and proteins are also well known to those of skill in the art, and are described in Section 5.11 above.

10 In instances wherein the compound to be administered is a peptide compound, DNA sequences encoding the peptide compound can be directly administered to a patient exhibiting immune disorder symptoms, at a concentration sufficient to produce a level of peptide compound sufficient to ameliorate
15 the disorder symptoms. Any of the techniques discussed, below, in Section 5.11, which achieve intracellular administration of compounds, such as, for example, liposome administration, can be utilized for the administration of such DNA molecules. The DNA molecules can be produced, for
20 example, by well known recombinant techniques.

In the case of peptides compounds which act extracellularly, the DNA molecules encoding such peptides can be taken up and expressed by any cell type, so long as a sufficient circulating concentration of peptide results for
25 the elicitation of a reduction in the immune disorder symptoms, or in a reduction of the symptoms of ischemic disorders and injuries. In the case of compounds which act intracellularly, the DNA molecules encoding such peptides must be taken up and expressed by the TH cell subpopulation
30 of interest at a sufficient level to bring about the reduction of immune disorders.

Any technique which serves to selectively administer DNA molecules to the TH cell subpopulation of interest is, therefore, preferred, for the DNA molecules encoding
35 intracellularly acting peptides. In the case of asthma, for example, techniques for the selective administration of the

molecules to TH cell subpopulations residing within lung tissue are preferred.

In cases wherein such molecules are administered to treat an ischemia related disorder or injury, preferred
5 techniques are those which serve to selectively administer DNA molecules to the affected organ or tissue. For example, in the case of ischemic renal disease, techniques for the selective administration of the molecules to cells within the kidney are preferred.

10 In instances wherein the TH cell subpopulation-related disorder involves an aberrant gene, patients can be treated by gene replacement therapy. One or more copies of a normal target gene or a portion of the gene that directs the
15 production of a normal target gene protein with target gene function, can be inserted into cells, using vectors which include, but are not limited to adenovirus, adeno-associated virus, and retrovirus vectors, in addition to other particles that introduce DNA into cells, such as liposomes.

Such gene replacement techniques can be accomplished
20 either in vivo or in vitro. As above, for genes encoding extracellular molecules, the cell type expressing the target gene is less important than achieving a sufficient circulating concentration of the extracellular molecule for the amelioration of immune disorders, or to ameliorate the
25 symptoms of ischemic disorders or injuries. Further, as above, when the gene encodes a cell which acts intracellularly or as a transmembrane molecule, the gene must be expressed with the TH cell subpopulation cell type of interest. Techniques which select for expression within the
30 cell type of interest are, therefore, preferred for this latter class of target genes. In vivo, such techniques can, for example, include appropriate local administration of target gene sequences.

Additional methods which may be utilized to increase the
35 overall level of target and/or pathway gene expression and/or target and/or pathway gene activity include the introduction of appropriate target and/or pathway gene-expressing cells,

preferably autologous cells, into a patient at positions and in numbers which are sufficient to ameliorate the symptoms of T cell subpopulation related disorders, or, alternatively, to ameliorate the symptoms of ischemic disorders or injuries.

5 Such cells may be either recombinant or non-recombinant.

Among the cells which can be administered to increase the overall level of target and/or pathway gene expression in a patient are normal cells, which express the target and/or pathway gene. The cells can be administered at the

10 anatomical site of expression, or as part of a tissue graft located at a different site in the body. Such cell-based gene therapy techniques are well known to those skilled in the art, see, e.g., Anderson, et al., U.S. Patent No.

5,399,349; Mulligan & Wilson, U.S. Patent No. 5,460,959.

15 In vitro, target gene sequences can be introduced into autologous cells. These cells expressing the target gene sequence of interest can then be reintroduced, preferably by intravenous administration, into the patient such that there results an amelioration of the symptoms of the disorder.

20 Alternatively, for the amelioration of a TH cell subpopulation-related disorder, TH cells belonging to a specific TH cell subpopulation can be administered to a patient such that the overall number of cells belonging to that TH cell subpopulation relative to other TH cell

25 subpopulation cells is increased. Techniques for such TH cell subpopulation augmentation are described, below, in Section 5.10.3.2.

5.10.3. NEGATIVE OR POSITIVE MODULATORY TECHNIQUES

30 Described herein are modulatory techniques which, depending on the specific application for which they are utilized, can yield either positive or negative responses leading to the amelioration of immune disorders, including TH cell subpopulation-related disorders. Further, the

35 modulatory techniques described in Section 5.10.3.1, below, can also be used to treat ischemic disorders and injuries, or, alternatively, to block or inhibit repair of ischemic

injuries depending on whether such modulation is positive or negative. Thus, in appropriate instances, the procedures of this Section can be used in conjunction with the negative modulatory techniques described, above, in Section 5.9.1 or, 5 alternatively, in conjunction with the positive modulatory techniques described, above, in Section 5.9.2.

5.10.3.1. ANTIBODY TECHNIQUES

Antibodies exhibiting modulatory capability can be 10 utilized to ameliorate immune disorders such as TH cell subpopulation-related disorders, or to treat ischemic disorders and injuries. Depending on the specific antibody, the modulatory effect can be negative and can, therefore, by utilized as part of the techniques described, above, in 15 Section 5.9.1, or can be positive, and can, therefore, be used in conjunction with the techniques described, above, in Section 5.9.2.

An antibody having negative modulatory capability refers to an antibody which specifically binds to and interferes 20 with the action of a protein. In the case of an extracellular receptor, for example, such an antibody would specifically bind the extracellular domain of the receptor in a manner which does not activate the receptor but which disrupts the ability of the receptor to bind its natural 25 ligand. For example, antibodies directed against the extracellular domains of genes 103 or 200 can function as such negative modulators. Additionally, antibodies directed against one or more of the 10 gene product extracellular domains can function in a negative modulatory manner. Such 30 antibodies can be generated using standard techniques described in Section 5.6, above, against full length wild type or mutant proteins, or against peptides corresponding to portions of the proteins. The antibodies include but are not limited to polyclonal, monoclonal, FAb fragments, single 35 chain antibodies, chimeric antibodies, and the like.

An antibody having positive modulatory capability refers to an antibody which specifically binds to a protein and, by

binding, serves to, either directly or indirectly, activate the function of the protein which it recognizes. For example, an antibody can bind to the extracellular portion of a transmembrane protein in a manner which causes the

5 transmembrane protein to function as though its endogenous ligand was binding, thus activating, for example, a signal transduction pathway. antibodies can be generated using standard techniques described in Section 5.6, above, against full length wild type or mutant proteins, or against peptides

10 corresponding to portions of the proteins. The antibodies include but are not limited to polyclonal, monoclonal, Fab fragments, single chain antibodies, chimeric antibodies, and the like.

In instances where the protein, such as a target gene

15 protein, to which the antibody is directed is intracellular and whole antibodies are used, internalizing antibodies can be preferred. However, lipofectin or liposomes can be used to deliver the antibody or a fragment of the Fab region which binds to the gene product epitope into cells. Where

20 fragments of the antibody are used, the smallest inhibitory fragment which binds to the protein's binding domain is preferred. For example, peptides having an amino acid sequence corresponding to the domain of the variable region of the antibody that binds to the protein can be used. Such

25 peptides can be synthesized chemically or produced via recombinant DNA technology using methods well known in the art (e.g., see Creighton, 1983, supra; and Sambrook et al., 1989, above). Alternatively, single chain antibodies, such as neutralizing antibodies, which bind to intracellular

30 epitopes can also be administered. Such single chain antibodies can be administered, for example, by expressing nucleotide sequences encoding single-chain antibodies within the target cell population by utilizing, for example, techniques such as those described in Marasco et al.

35 (Marasco, W. et al., 1993, Proc. Natl. Acad. Sci. USA 90:7889-7893).

In instances where the protein to which the antibody is directed is extracellular, or is a transmembrane protein, any of the administration techniques described, below in Section 5.11 which are appropriate for peptide administration can be utilized to effectively administer the antibodies to their site of action.

5.10.3.2. METHODS FOR INCREASING OR DECREASING SPECIFIC TH CELL SUBPOPULATION CONCENTRATIONS

10 Techniques described herein can be utilized to either deplete or augment the total number of cells belonging to a given TH cell subpopulation, thus effectively increasing or decreasing the ratio of the TH cell subpopulation of interest to other TH cell subpopulations. Specifically, separation
15 techniques are described which can be used to either deplete or augment the total number of cells present within a TH cell subpopulation, and, further, targeting techniques are described which can be utilized to deplete specific TH cell subpopulations.

20 Depending on the particular application, changing the number of cells belonging to a TH cell subpopulation can yield either stimulatory or inhibitory responses leading to the amelioration of TH cell subpopulation disorders. Thus, in appropriate instances, the procedures of this Section can
25 be used in conjunction with the inhibitory techniques described, above, in Section 5.9.1. or, alternatively, in conjunction with the stimulatory techniques described, above, in Section 5.9.2.

The separation techniques described herein are based on
30 the presence or absence of specific cell surface markers, preferably transmembrane markers. Such markers can include, but are not limited to, the TH2-specific 103 gene product extracellular domain markers, the TH1-specific 200 gene product extracellular domain markers and the TH inducible 10
35 gene product extracellular domain markers.

In instances wherein the goal of the separation is to increase or augment the number of cells belonging to a

specific TH cell subpopulation, the antibodies used can also be specific to surface markers present on undifferentiated or partially undifferentiated TH cells. After separation, and purification of such undifferentiated or partially

5 differentiated TH cells, the cells can be cultured in physiological buffer or culture medium and induced to differentiate by culturing in the presence of appropriate factors. For example, IL-4 can be added to induce the TH cells to differentiate into TH2 cells, while the cytokine IL-

10 12 can be added to induce the TH cells to differentiate into TH1 cells. After differentiation, cells can be washed, resuspended in, for example, buffered saline, and reintroduced into a patient via, preferably, intravenous administration.

15 Separation techniques can be utilized which separate and purify cells, in vitro, from a population of cells, such as hematopoietic cells autologous to the patient being treated. An initial TH cell subpopulation-containing population of cells, such as hematopoietic cells, can be obtained using

20 standard procedures well known to those of skill in the art. Peripheral blood can be utilized as one potential starting source for such techniques, and can, for example, be obtained via venipuncture and collection into heparinized tubes.

Once the starting source of autologous cells is

25 obtained, the T cells, such as TH1 or TH2 cells, can be removed, and thus selectively separated and purified, by various methods which utilize antibodies which bind specific markers present on the T cell population of interest, while absent on other cells within the starting source. These

30 techniques can include, for example, flow cytometry using a fluorescence activated cell sorter (FACS) and specific fluorochromes, biotin-avidin or biotin-streptavidin separations using biotin conjugated to cell surface marker-specific antibodies and avidin or streptavidin bound to a

35 solid support such as affinity column matrix or plastic surfaces or magnetic separations using antibody-coated magnetic beads.

Separation via antibodies for specific markers can be by negative or positive selection procedures. In negative separation, antibodies are used which are specific for markers present on undesired cells. For example, in the case of a TH1 cell subpopulation-related disorder wherein it would be desirable to deplete the number of TH1 cells, such antibodies could be directed to the extracellular domain of the 200 gene product. Alternatively, in the case of TH2 cell subpopulation-related disorders wherein it would be desirable to deplete the number of TH1 cells, such antibodies could be directed to the extracellular domain of the 103 gene product. Cells bound by an antibody to such a cell surface marker can be removed or lysed and the remaining desired mixture retained.

In positive separation, antibodies specific for markers present on the desired cells of interest. For example, in the case of a TH1 cell subpopulation-related disorder wherein it would be desirable to increase the number of TH1 cells, such antibodies could be directed to the extracellular domain of the 200 gene product. Alternatively, in the case of TH2 cell subpopulation-related disorders wherein it would be desirable to increase the number of TH1 cells, such antibodies could be directed to the extracellular domain of the 103 gene product. Cells bound by the antibody are separated and retained. It will be understood that positive and negative separations can be used substantially simultaneously or in a sequential manner.

A common technique for antibody based separation is the use of flow cytometry such as by a fluorescence activated cell sorter (FACS). Typically, separation by flow cytometry is performed as follows. The suspended mixture of cells are centrifuged and resuspended in media. Antibodies which are conjugated to fluorochrome are added to allow the binding of the antibodies to specific cell surface markers. The cell mixture is then washed by one or more centrifugation and resuspension steps. The mixture is run through a FACS which separates the cells based on different fluorescence

characteristics. FACS systems are available in varying levels of performance and ability, including multi-color analysis. The facilitating cell can be identified by a characteristic profile of forward and side scatter which is
5 influenced by size and granularity, as well as by positive and/or negative expression of certain cell surface markers.

Other separation techniques besides flow cytometry can also provide fast separations. One such method is biotin-avidin based separation by affinity chromatography.

10 Typically, such a technique is performed by incubating cells with biotin-coupled antibodies to specific markers, such as, for example, the transmembrane protein encoded by the 103 gene described herein, followed by passage through an avidin column. Biotin-antibody-cell complexes bind to the column
15 via the biotin-avidin interaction, while other cells pass through the column. The specificity of the biotin-avidin system is well suited for rapid positive separation. Multiple passages can ensure separation of a sufficient level of the TH cell subpopulation of interest.

20 In instances whereby the goal of the separation technique is to deplete the overall number of cells belonging to a TH cell subpopulation, the cells derived from the starting source of cells which has now been effectively depleted of TH cell subpopulation cells can be reintroduced
25 into the patient. Such a depletion of the TH cell subpopulation results in the amelioration of TH cell subpopulation-related disorders associated with the activity or overactivity of the TH cell subpopulation. Reintroduction of the TH cell subpopulation-depleted cells can be
30 accomplished by washing the cells, resuspending in, for example, buffered saline, and intravenously administering the cells into the patient.

If cell viability and recovery are sufficient, TH cell subpopulation-depleted cells can be reintroduced into
35 patients immediately subsequent to separation. Alternatively, TH cell subpopulation-depleted cells can be cultured and expanded ex vivo prior to administration to a

patient. Expansion can be accomplished via well known techniques utilizing physiological buffers or culture media in the presence of appropriate expansion factors such as interleukins and other well known growth factors.

5 In instances whereby the goal of the separation technique is to augment or increase the overall number of cells belonging to a TH cell subpopulation, cells derived from the purified TH cell subpopulation cells can be reintroduced into the patient, thus resulting in the
10 amelioration of TH cell subpopulation-related disorders associated with an under activity of the TH cell subpopulation.

The cells to be reintroduced will be cultured and expanded ex vivo prior to reintroduction. Purified TH cell
15 subpopulation cells can be washed, suspended in, for example, buffered saline, and reintroduced into the patient via intravenous administration.

Cells to be expanded can be cultured, using standard procedures, in the presence of an appropriate expansion agent
20 which induces proliferation of the purified TH cell subpopulation. Such an expansion agent can, for example, be any appropriate cytokine, antigen, or antibody. In the case of TH2 cells, for example, the expansion agent can be IL-4, while for TH1 cells, the expansion agent can, for example, be
25 IL-12.

Prior to being reintroduced into a patient, the purified cells can be modified by, for example, transformation with gene sequences encoding gene products of interest. Such gene products should represent products which enhance the activity
30 of the purified TH cell subpopulation or, alternatively, represent products which repress the activity of one or more of the other TH cell subpopulations. Cell transformation and gene expression procedures are well known to those of skill in the art, and can be as those described, above, in Section
35 5.5.

Well known targeting methods can, additionally, be utilized in instances wherein the goal is to deplete the

number of cells belonging to a specific TH cell subpopulation. Such targeting methods can be in vivo or in vitro, and can involve the introduction of targeting agents into a population of cells such that the targeting agents
5 selectively destroy a specific subset of the cells within the population. In vivo administration techniques which can be followed for such targeting agents are described, below, in Section 5.11.

Targeting agents generally comprise, first, a targeting
10 moiety which, in the current instance, causes the targeting agent to selectively associate with a specific TH cell subpopulation. The targeting agents generally comprise, second, a moiety capable of destroying a cell with which the targeting agent has become associated.

15 Targeting moieties can include, but are not limited to, antibodies directed to cell surface markers found specifically on the TH cell subpopulation being targeted, or, alternatively, to ligands, such as growth factors, which bind receptor-type molecules found exclusively on the targeted TH
20 cell subpopulation.

In the case of TH2 cells, for example, such a targeting moiety can represent an antibody directed against the extracellular portion of the 103 gene product described herein, or can, alternatively, represent a ligand specific
25 for this receptor-type TH2 specific molecule. In the case of TH1 cells, for example, such a targeting moiety can represent an antibody directed against the extracellular portion of the 200 gene product described herein, or can, alternatively, represent a ligand specific for this receptor-type TH1
30 specific molecule.

Destructive moieties include any moiety capable of inactivating or destroying a cell to which the targeting agent has become bound. For example, a destructive moiety can include, but it is not limited to cytotoxins or
35 radioactive agents. Cytotoxins include, for example, plant-, fungus-, or bacteria-derived toxins, with deglycosylated

Ricin A chain toxins being generally preferred due to their potency and lengthy half-lives.

5.11. PHARMACEUTICAL PREPARATIONS AND METHODS OF ADMINISTRATION

5 The compounds, nucleic acid sequences and TH cell subpopulation cell described herein can be administered to a patient at therapeutically effective doses to treat or ameliorate immune disorders, e.g., TH cell subpopulation-related disorders. A therapeutically effective dose refers to that amount of a compound or TH cell subpopulation sufficient to result in amelioration of the immune disorder symptoms of the immune disorder symptoms, or alternatively, to that amount of a nucleic acid sequence sufficient to express a concentration of gene product which results in the amelioration of the TH cell subpopulation-related disorders or of other immune disorders.

5.11.1. EFFECTIVE DOSE

20 Toxicity and therapeutic efficacy of compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

35 The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage can

vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially
5 from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information
10 can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of antibody, protein, or polypeptide (i.e., an effective dose or
15 effective dosage) ranges from about 0.001 to 30 mg/kg of body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight.

20 The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present.
25 Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide or antibody can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with antibody, protein, or polypeptide in the range
30 of between about 0.1 to 20 mg/kg body weight one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody, protein or
35 polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may

also be apparent to one skilled in the art from the results of diagnostic assays as described herein.

5.11.2. FORMULATIONS AND USE

- 5 Pharmaceutical compositions for use in accordance with the present invention can be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.
- Thus, the compounds and their physiologically acceptable
10 salts and solvents can be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral, subcutaneous, intraperitoneal, intrapulmonary, intranasal, intralesional, vaginal or rectal administration.
- 15 For oral administration, the pharmaceutical compositions can take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone or
20 hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate).
- 25 The tablets can be coated by methods well known in the art. Liquid preparations for oral administration can take the form of, for example, solutions, syrups or suspensions, or they can be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid
30 preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily
35 esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations can also contain buffer

salts, flavoring, coloring and sweetening agents as appropriate.

Preparations for oral administration can be suitably formulated to give controlled release of the active compound.

- 5 For buccal administration the compositions can take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered
10 in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit
15 can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator can be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

- 20 The compounds can be formulated for parenteral administration (i.e., intravenous or intramuscular) by injection, via, for example, bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, e.g., in ampoules or in multi-dose
25 containers, with an added preservative. The compositions can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient can be in powder form
30 for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use. It is preferred that the TH cell subpopulation cells be introduced into patients via intravenous administration.

The compounds can also be formulated in rectal
35 compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds can also be formulated as a depot preparation. Such long acting formulations can be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds can be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Intralesional administration can comprise, for example, perfusing or contacting a graft or organ with a composition prior to transplantation.

The compositions can, if desired, be presented in a pack or dispenser device which can contain one or more unit dosage forms containing the active ingredient. The pack can for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device can be accompanied by instructions for administration.

20

5.11.3. PHARMACEUTICAL PREPARATION AND ADMINISTRATION OF ANTIBODIES

Antibodies which specifically bind to target gene products of the invention and thereby modulate their activity can also be administered to a patient at therapeutically effective doses to treat or ameliorate immune disorders, or to treat or ameliorate ischemic disorders or injuries. For example, Section 13, below, demonstrates the use of anti-200 gene product antibodies to block recovery of kidney tissue from ischemia reperfusion injury.

Antibodies of the invention are administered by any suitable means, including those described in Section 5.12.2, above. In addition, antibody to a target gene product of the invention is suitably administered by pulse infusion, particularly with declining doses of the antibody. Preferably, the dosing is administered by injections, most preferably by intravenous or subcutaneous injections,

depending in part on whether the administration is brief or chronic.

The appropriate dosage of antibody will depend on the type of disease to be treated, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody may suitably administered to the patient at one time or, more preferably, over a series of treatments.

As a general proposition, the initial pharmaceutically effective amount of antibody administered parenterally will be in the range of about 0.1 to 20 mg/kg of patient body weight per day, with the typical initial range being 0.3 to 15 mg/kg of patient body weight per day. Where the subsequent dosing is less than 100% of initial dosing, such subsequent dosing is calculated on the basis of daily dosing. Thus, for example, if the dosing regimen consists of daily injections of 2 mg/kg of patient body weight per day for two weeks followed by a biweekly dose of 0.5 mg/kg of patient body weight per day for 99 days, this would amount to a subsequent dose of about 1.8% of the initial dose calculated on a daily basis (i.e., $2/\text{day}/100\% = 0.5/14 \text{ days}/x\%$, $x=1.8\%$). Preferably, the subsequent dosing is less than about 50%, more preferably, less than about 25%, still more preferably, less than about 10%, and still more preferably, less than about 5%. Most preferably, the subsequent dosing is less than about 2% of the initial dosing.

Overall, dosage ranges to be administered will, preferably, range from about 1 $\mu\text{g}/\text{kg}$ to about 100 mg/kg, 1 $\mu\text{g}/\text{kg}$ to about 15 mg/kg, or about 1 $\mu\text{g}/\text{kg}$, to about 2.0 mg/kg body weight.

The preferred scheduling is that the initial dosing is administered no less frequently than daily, and up to an including continuously by infusion. More preferably, depending on the specific disorder or injury, the initial daily dosing is administered for at least about one week, and

preferably at least about two weeks. To obtain the most efficacious results, the initial dosing is preferably given as close to the first sign, diagnosis, appearance, or occurrence of the disorder as possible, or, particular in the case of
5 immune disorders, during remission of the disorder.

Subsequent dosing is preferably administered periodically no more about once a week. More preferably, the subsequent dosing is administered no more than once biweekly. Subsequent dosing is typically administered for at least
10 about five weeks, and preferably for at least about 10 weeks, after the initial dosing is terminated.

Exemplary dosing regimens are disclosed in Jardieu, P.M., and Presta, L.G., 1998, WO 98/23761; and in Jardieu, P.M., and Presta, L.G., 1994, WO 94/04188, each of which is
15 incorporated herein, by reference, in its entirety.

5.12. DIAGNOSTIC AND MONITORING TECHNIQUES

A variety of methods can be employed for the diagnosis of immune disorders, e.g., TH cell subpopulation-related
20 disorders, predisposition to such immune disorders, for monitoring the efficacy of anti-immune disorder compounds during, for example, clinical trials and for monitoring patients undergoing clinical evaluation for the treatment of such disorders. Further, a number of methods can be utilized
25 for the detection of activated immune cells, e.g., activated members of TH cell subpopulations.

Such methods can, for example, utilize reagents such as the fingerprint gene nucleotide sequences described in Sections 5.1, and antibodies directed against differentially
30 expressed and pathway gene peptides, as described, above, in Sections 5.5 (peptides) and 5.6 (antibodies). Specifically, such reagents can be used, for example, for: 1) the detection of the presence of target gene expression, target gene mutations, the detection of either over- or under-expression
35 of target gene mRNA relative to the non-immune disorder state or relative to an unactivated TH cell subpopulation; 2) the detection of either an over- or an underabundance of target

gene product relative to the non-immune disorder state or relative to the unactivated TH cell subpopulation state; and 3) the identification of specific TH cell subpopulation cells (e.g., TH cells involved in an immune disorder, or activated 5 TH cells) within a mixed population of cells.

The methods described herein can be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one specific fingerprint gene nucleic acid or anti-fingerprint gene antibody reagent described herein, which can 10 be conveniently used, e.g., in clinical settings, to diagnose patients exhibiting TH1- or TH2-related abnormalities.

Any cell type or tissue, preferably TH cells, in which the fingerprint gene is expressed can be utilized in the diagnostics described below.

15 Among the methods which can be utilized herein are methods for monitoring the efficacy of compounds in clinical trials for the treatment of immune disorders. Such compounds can, for example, be compounds such as those described, above, in Section 5.9. Such a method comprises detecting, in 20 a patient sample, a gene transcript or gene product which is differentially expressed in a TH cell subpopulation in an immune disorder state relative to its expression in the TH cell subpopulation when the cell subpopulation is in a normal, or non-immune disorder, state.

25 Any of the nucleic acid detection techniques described, below, in Section 5.12.1 or any of the peptide detection techniques described, below, in Section 5.12.2 can be used to detect the gene transcript or gene product which is differentially expressed in the immune disorder TH cell 30 subpopulation relative to its expression in the normal, or non-immune disorder, state.

During clinical trials, for example, the expression of a single fingerprint gene, or alternatively, the fingerprint pattern of a TH cell subpopulation, can be determined for the 35 TH cell subpopulation in the presence or absence of the compound being tested. The efficacy of the compound can be followed by comparing the expression data obtained to the

corresponding known expression patterns for the TH cell subpopulation in a normal, non-immune disorder state.

Compounds exhibiting efficacy are those which alter the single fingerprint gene expression and/or the fingerprint
5 pattern of the immune disorder TH cell subpopulation to more closely resemble that of the normal, non-immune disorder TH cell subpopulation.

The detection of the product or products of genes differentially expressed in a TH cell subpopulation in an
10 immune disorder state relative to their expression in the TH cell subpopulation when the cell subpopulation is in a normal, or non-immune disorder, state can also be used for monitoring the efficacy of potential anti-immune disorder compounds during clinical trials. During clinical trials,
15 for example, the level and/or activity of the products of one or more such differentially expressed genes can be determined for the TH cell subpopulation in the presence or absence of the compound being tested. The efficacy of the compound can be followed by comparing the protein level and/or activity
20 data obtained to the corresponding known levels/activities for the TH cell subpopulation in a normal, non-immune disorder state. Compounds exhibiting efficacy are those which alter the pattern of the immune disorder TH cell subpopulation to more closely resemble that of the normal,
25 non-immune disorder TH cell subpopulation.

Given the TH2-specific nature of the 103 gene, the detection of 103 gene transcripts and/or products can be particularly suitable for monitoring the efficacy of compounds in clinical trials for the treatment of TH2 cell
30 subpopulation-related immune disorders such as, for example, asthma or allergy.

The expression patterns of the 105, 106 and 200 genes in TH1 cell subpopulations relative to TH2 cell subpopulations can make the detection of transcripts and/or products of
35 these genes particularly suitable for monitoring the efficacy of compounds in clinical trials for the treatment of TH1 cell

subpopulation-related immune disorders such as, for example, multiple sclerosis, psoriasis or insulin dependent diabetes.

Among the additional methods which can be utilized herein are methods for detecting TH cell responsiveness, for example, responsiveness to antigen, and for detecting activated immune cells, e.g., activated members of TH cell subpopulations. Detection methods such as these are important in that many immune disorders involve inappropriate rather than insufficient immune responses. Such detection methods can be used, for example, to detect a predisposition to an immune disorder.

Methods for detecting TH cell responsiveness and/or activation can comprise, for example, detecting in a TH cell sample a gene transcript or product which is differentially expressed in TH cell subpopulation which is in an activated or responsive state (e.g., a state in which the TH cell subpopulation has been exposed to antigen), relative to a TH cell subpopulation which is in an unactivated or nonresponsive state.

Any of the nucleic acid detection techniques described, below, in Section 5.12.1 or any of the peptide detection techniques described, below, in Section 5.12.2 can be used to detect such a differentially expressed gene transcript or gene product.

In addition to diagnostic uses, such techniques can also be utilized as part of methods for identifying compounds which alter the cellular expression of one or more of the differentially expressed genes described herein, or as part of methods for identifying compounds which alter the cellular and/or secreted level of product produced by the differentially expressed genes described herein.

By way of example, and not by way of limitation, such techniques can be used to identify compounds which alter the level of expression of the 103 gene or the level of 103 gene product present in a cell. Such methods can include, for example, contacting a T cell with a compound, measuring the level of 103 gene expression in the cell (or the level of 103

gene product in the cell), then comparing the level obtained to that of a cell not exposed to the compound. The T cells used herein can include, for example, TH0, TH1 or TH2 cells.

Such methods can further include stimulating the cells, 5 for example, stimulating the cells prior to contacting the cells with the compound. Among the methods for stimulation are stimulation via anti-CD3 antibody stimulation.

Such methods can be performed such that the cell contacted is presented within a non-human mammal, for 10 example, a mouse. Further, among the non-human mammals which can be utilized as part of these methods are ones which exhibit symptoms of a T cell-related disorder (such as, for example a TH2-related disorder, e.g., asthma), and contacting the cell with the compound can ameliorate symptoms of the 15 disorder.

The TH2-specific nature of the 103 gene can make the detection of its gene transcripts and/or products particularly suitable for detecting activation and/or responsiveness of TH2 cells. Further, the TH1-specific 20 nature of the 105, 106 and 200 genes can make the detection of transcripts and/or products of these genes particularly suitable for the detection of TH1 activation and/or responsiveness.

25 5.12.1. DETECTION OF FINGERPRINT GENE NUCLEIC ACIDS

DNA or RNA from the cell type or tissue to be analyzed can easily be isolated using procedures which are well known to those in the art. Diagnostic procedures can also be performed "in situ" directly upon, for example tissue 30 sections (fixed and/or frozen) of patient tissue obtained from biopsies or resections, such that no nucleic acid purification is necessary. Nucleic acid reagents such as those described in Section 5.4 can be used as probes and/or primers for such in situ procedures (see, for example, Nuovo, 35 G.J., 1992, "PCR In Situ Hybridization: Protocols and Applications", Raven Press, NY). Expression of specific cells within a population of cells can also be determined,

via, for example, in situ techniques such as those described above, or by standard flow cytometric techniques.

Fingerprint gene nucleotide sequences, either RNA or DNA, can, for example, be used in hybridization or
5 amplification assays of biological samples to detect TH cell subpopulation-related disorder gene structures and expression. Such assays can include, but are not limited to, Southern or Northern analyses, single stranded conformational polymorphism analyses, in situ hybridization assays, and
10 polymerase chain reaction analyses. Such analyses can reveal both quantitative aspects of the expression pattern of the fingerprint gene, and qualitative aspects of the fingerprint gene expression and/or gene composition. That is, such techniques can detect not only the presence of gene
15 expression, but can also detect the amount of expression, particularly which specific cells are expressing the gene of interest, and can, further, for example, detect point mutations, insertions, deletions, chromosomal rearrangements, and/or activation or inactivation of gene expression.
20 Diagnostic methods for the detection of fingerprint gene-specific nucleic acid molecules can involve for example, contacting and incubating nucleic acids, derived from the cell type or tissue being analyzed, with one or more labeled nucleic acid reagents as are described in Section 5.4, under
25 conditions favorable for the specific annealing of these reagents to their complementary sequences within the nucleic acid molecule of interest. Preferably, the lengths of these nucleic acid reagents are at least 15 to 30 nucleotides. After incubation, all non-annealed nucleic acids are removed
30 from the nucleic acid:fingerprint molecule hybrid. The presence of nucleic acids from the cell type or tissue which have hybridized, if any such molecules exist, is then detected. Using such a detection scheme, the nucleic acid from the tissue or cell type of interest can be immobilized,
35 for example, to a solid support such as a membrane, or a plastic surface such as that on a microtiter plate or polystyrene beads. In this case, after incubation, non-

annealed, labeled nucleic acid reagents of the type described in Section 5.4 are easily removed. Detection of the remaining, annealed, labeled fingerprint nucleic acid reagents is accomplished using standard techniques well-known to those in the art.

Alternative diagnostic methods for the detection of fingerprint gene specific nucleic acid molecules can involve their amplification, e.g., by PCR (the experimental embodiment set forth in Mullis, K.B., 1987, U.S. Patent No. 4,683,202), ligase chain reaction (Barany, F., 1991, Proc. Natl. Acad. Sci. USA 88:189-193), self sustained sequence replication (Guatelli, J.C. et al., 1990, Proc. Natl. Acad. Sci. USA 87:1874-1878), transcriptional amplification system (Kwoh, D.Y et al., 1989, Proc. Natl. Acad. Sci. USA 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. et al., 1988, Bio/Technology 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In one embodiment of such a detection scheme, a cDNA molecule is obtained from an RNA molecule of interest (e.g., by reverse transcription of the RNA molecule into cDNA). Cell types or tissues from which such RNA can be isolated include any tissue in which wild type fingerprint gene is known to be expressed, including, but not limited, to TH0, TH1 and/or TH2 cell type-containing tissues. A sequence within the cDNA is then used as the template for a nucleic acid amplification reaction, such as a PCR amplification reaction, or the like. The nucleic acid reagents used as synthesis initiation reagents (e.g., primers) in the reverse transcription and nucleic acid amplification steps of this method are chosen from among the fingerprint gene nucleic acid reagents described in Section 5.4. The preferred lengths of such nucleic acid reagents are at least 9-30 nucleotides. For detection of the amplified product, the

nucleic acid amplification can be performed using radioactively or non-radioactively labeled nucleotides. Alternatively, enough amplified product can be made such that the product can be visualized by standard ethidium bromide staining or by utilizing any other suitable nucleic acid staining method.

In addition to methods which focus primarily on the detection of one fingerprint nucleic acid sequence, fingerprint patterns can also be assessed in such detection schemes. Fingerprint patterns, in this context, contain the pattern of mRNA expression of a series (i.e., at least two and up to the total number present) of fingerprint genes obtained for a given tissue or cell type under a given set of conditions. Such conditions can include, for example, TH cell subpopulation-related disorders, and conditions relevant to processes involved in the differentiation, maintenance and effector function of TH cell subpopulations.

TH1-related disorders can include, for example, chronic inflammatory diseases and disorders, such as Crohn's disease, reactive arthritis, including Lyme disease, insulin-dependent diabetes, organ-specific autoimmunity, including multiple sclerosis, Hashimoto's thyroiditis and Grave's disease, contact dermatitis, psoriasis, graft rejection, graft versus host disease and sarcoidosis. TH2-related disorders can include, for example, atopic conditions, such as asthma and allergy, including allergic rhinitis, gastrointestinal allergies, including food allergies, eosinophilia, conjunctivitis, glomerular nephritis, certain pathogen susceptibilities such as helminthic (e.g., leishmaniasis) and certain viral infections, including HIV, and bacterial infections, including tuberculosis and lepromatous leprosy.

Fingerprint patterns can be generated, for example, by utilizing a differential display procedure, as discussed, above, in Section 5.1.1.2, Northern analysis and/or RT-PCR. Any of the gene sequences described, above, in Section 3.2.1 can be used as probes and/or RT-PCR primers for the generation and corroboration of such fingerprint patterns.

5.12.2. DETECTION OF TARGET GENE PEPTIDES

Antibodies directed against wild type or mutant fingerprint gene peptides, which are discussed, above, in Section 5.6, can also be used as TH cell subpopulation-

5 related disorder diagnostics and prognostics, as described, for example, herein. Such diagnostic methods, can be used to detect fingerprint gene product, abnormalities in the level of fingerprint gene protein expression, or abnormalities in the structure and/or temporal, tissue, cellular, or
10 subcellular location of fingerprint gene protein. Structural differences can include, for example, differences in the size, electronegativity, or antigenicity of the mutant fingerprint gene protein relative to the normal fingerprint gene protein.

15 Protein from the tissue or cell type to be analyzed can easily be isolated using techniques which are well known to those of skill in the art. The protein isolation methods employed herein can, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988,
20 "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), which is incorporated herein by reference in its entirety.

Preferred diagnostic methods for the detection of wild type or mutant fingerprint gene peptide molecules can
25 involve, for example, immunoassays wherein fingerprint gene peptides are detected by their interaction with an anti-fingerprint gene product-specific antibody.

For example, antibodies, or fragments of antibodies, such as those described, above, in Section 5.6, useful in the
30 present invention can be used to quantitatively or qualitatively detect the presence of wild type or mutant fingerprint gene peptides. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody (see below, this Section,)
35 coupled with light microscopic, flow cytometric, or fluorimetric detection. Such techniques are especially preferred if the fingerprint gene peptides are expressed on

the cell surface, such as, for example, is the case with the 10 gene product, the 200 gene product and the transmembrane form of 103 gene product. Thus, the techniques described herein can be used to detect specific cells, within a
5 population of cells, which express the fingerprint gene product of interest.

The antibodies (or fragments thereof) useful in the present invention can, additionally, be employed histologically, as in immunofluorescence or immunoelectron
10 microscopy, for in situ detection of fingerprint gene peptides. In situ detection can be accomplished by removing a histological specimen from a patient, and applying thereto a labeled antibody of the present invention. The antibody (or fragment) is preferably applied by overlaying the labeled
15 antibody (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the fingerprint gene peptides, but also their distribution in the examined tissue. Using the present invention, those of ordinary skill will readily perceive that
20 any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such in situ detection.

Immunoassays for wild type or mutant fingerprint gene peptides typically comprise incubating a biological sample,
25 such as a biological fluid, a tissue extract, freshly harvested cells, or cells which have been incubated in tissue culture, in the presence of a detectably labeled antibody capable of identifying fingerprint gene peptides, and detecting the bound antibody by any of a number of techniques
30 well-known in the art.

The biological sample can be brought in contact with and immobilized onto a solid phase support or carrier such as nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble proteins. The
35 support can then be washed with suitable buffers followed by treatment with the detectably labeled fingerprint gene-specific antibody. The solid phase support can then be

washed with the buffer a second time to remove unbound antibody. The amount of bound label on solid support can then be detected by conventional means.

By "solid phase support or carrier" is intended any support capable of binding an antigen or an antibody. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material can have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration can be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface can be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

The binding activity of a given lot of anti-wild type or mutant fingerprint gene product antibody can be determined according to well known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

One of the ways in which the fingerprint gene peptide-specific antibody can be detectably labeled is by linking the same to an enzyme and use in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)", 1978, Diagnostic Horizons 2:1-7, Microbiological Associates Quarterly Publication, Walkersville, MD); Voller, A. et al., 1978, J. Clin. Pathol. 31:507-520; Butler, J.E., 1981, Meth. Enzymol. 73:482-523; Maggio, E. (ed.), 1980, ENZYME IMMUNOASSAY, CRC Press, Boca Raton, FL; Ishikawa, E. et al., (eds.), 1981, ENZYME IMMUNOASSAY, Kigaku Shoin, Tokyo). The

enzyme which is bound to the antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or
5 by visual means. Enzymes which can be used to detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase,
10 horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. The detection can be accomplished by colorimetric methods which employ a chromogenic substrate for
15 the enzyme. Detection can also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

Detection can also be accomplished using any of a variety of other immunoassays. For example, by radioactively
20 labeling the antibodies or antibody fragments, it is possible to detect fingerprint gene wild type or mutant peptides through the use of a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The
25 Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by such means as the use of a gamma counter or a scintillation counter or by autoradiography.

It is also possible to label the antibody with a
30 fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wavelength, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin,
35 phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

The antibody can also be detectably labeled using fluorescence emitting metals such as ¹⁵²Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as

5 diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

The antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by
10 detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

15 Likewise, a bioluminescent compound can be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent
20 protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

6. EXAMPLE: IDENTIFICATION AND CHARACTERIZATION OF A TH2-
25 ENRICHED GENE

In the Example presented in this Section, the transgenic T cell paradigm described, above, in Section 5.1.1.1, , was utilized to identify a gene, designated herein as the 102 gene, which is expressed in TH2 cells. The identified gene
30 is present in TH2 cells at a much higher level than in TH1 cells. Thus, the Example presented herein demonstrates the usefulness of the paradigm approach of the invention for the identification of genes that are differentially expressed in TH cell subpopulations.

35

6.1. MATERIALS AND METHODS

Transgenic mice: Naive CD4⁺ cells were obtained from the spleens and/or lymph nodes of unprimed transgenic mouse strains harboring a T cell receptor (TCR) recognizing 5 ovalbumin (Murphy et al., 1990, Science 250:1720).

Ova-specific transgenic T cells: Suspensions of ova-specific T cells were co-cultured with stimulatory peptide antigen and antigen presenting cells essentially as described 10 in Murphy et al. (Murphy et al., 1990, Science 250:1720). Briefly, $2-4 \times 10^6$ T cells were incubated with approximately twice as many TA3 antigen presenting cells in the presence of 0.3 μ M Ova peptide. TH1 cultures contained approximately 10 ng/ml recombinant mIL-12. Conversely, TH2 cells received IL- 15 4 (1000u/ml). Cultures were harvested at various time points after initiation of culture. T cells were purified of TA3 cells using anti-CD4 coated magnetic beads (Dynal, Inc.). T cells were pelleted by gentle centrifugation and lysed in the appropriate volume of RNazol™ (Tel-Test, Friendswood, TX).

20

Tissue collection and RNA isolation: Cells were quick frozen on dry ice. Samples were then homogenized together with a mortar and pestle under liquid nitrogen.

Total cellular RNA was extracted from tissue with either 25 RNazol™ or RNazolB™ (Tel-Test, Friendswood, TX), according to the manufacturer's instructions. Briefly, the tissue was solubilized in an appropriate amount of RNazol™ or RNazolB™, and RNA was extracted by the addition of 1/10 v/v chloroform to the solubilized sample followed by vigorous shaking for 30 approximately 15 seconds. The mixture was then centrifuged for 15 minutes at 12,000g and the aqueous phase was removed to a fresh tube. RNA was precipitated with isopropanol. The resultant RNA pellet was dissolved in water and re-extracted with an equal volume of chloroform to remove any remaining 35 phenol. The extracted volume was precipitated with 2 volumes of ethanol in the presence of 150mM sodium acetate. The

precipitated RNA was dissolved in water and the concentration determined spectroscopically (A_{260}).

- Differential display: Total cellular RNA (10-50 μ g)
5 was treated with 20 Units DNase I (Boehringer Mannheim, Germany) in the presence of 40 Units ribonuclease inhibitor (Boehringer Mannheim, Germany). After extraction with phenol/chloroform and ethanol precipitation, the RNA was dissolved in DEPC (diethyl pyrocarbonate)-treated water.
- 10 Differential mRNA display was carried out as described, above, in Section 5.1.1.2. RNA (0.4-2 μ g) was reverse-transcribed using Superscript reverse transcriptase (GIBCO/BRL). The cDNAs were then amplified by PCR on a Perkin-Elmer 9600 thermal cycler. The reaction mixtures
15 (20 μ l) included arbitrary decanucleotides and one of twelve possible T_{11} VN sequences, wherein V represents either dG, dC, or dA, and N represents either dG, dT, dA, or dC. Parameters for the 40 cycle PCR were as follows: Hold 94° C. 2 minutes; Cycle 94° C. 15 seconds, 40° C. 2 minutes; Ramp to 72° 30
20 seconds; Hold 72° C. 5 minutes; Hold 4° C.

Radiolabelled PCR amplification products were analyzed by electrophoresis on 6% denaturing polyacrylamide gels.

- Reamplification and subcloning: PCR bands of interest
25 were recovered from sequencing gels and reamplified.

Briefly, autoradiograms were aligned with the dried gel, and the region containing the bands of interest was excised with a scalpel. The excised gel fragment was eluted by soaking in 100 μ l TE (Tris-EDTA) buffer at approximately 100°
30 C. for 15 minutes. The gel slice was then pelleted by brief centrifugation and the supernatant was transferred to a new microcentrifuge tube. DNA was combined with ethanol in the presence of 100mM Sodium acetate and 30 μ g glycogen
(Boehringer Mannheim, Germany) and precipitated on dry ice
35 for approximately 10 minutes. Samples were centrifuged for 10 minutes and pellets were washed with 80% ethanol. Pellets were resuspended in 10 μ l distilled water.

5 μ l of the eluted DNA were reamplified in a 100 μ l reaction containing: standard Cetus Taq polymerase buffer, 20 μ M dNTPs, 1 μ M of each of the oligonucleotide primers used in the initial generation of the amplified DNA. Cycling
5 conditions used were the same as the initial conditions used to generate the amplified band, as described above. One-half of the amplification reaction was run on a 2% agarose gel and eluted using DE-81 paper (Whatman Paper, Ltd., England) as described in Sambrook et al., supra. Recovered fragments
10 were ligated into the cloning vector pCR[™]II (Invitrogen, Inc., San Diego CA) and transformed into competent E. coli strain DH5 α (Gibco/BRL, Gaithersburg, MD). Colonies were grown on LB-agar plates containing ampicillin (100 μ g/ml) and X-gal (40 μ g/ml) to permit blue/white selection.

15

Sequence analysis: After subcloning, reamplified cDNA fragments were sequenced on an Applied Biosystems Automated Sequencer (Applied Biosystems, Inc. Seattle, WA). Sequence was obtained from four or more independent transformants
20 containing the same insert. The nucleotide sequence shown herein represents either the consensus of the information obtained from the four sequences, or the sequence obtained from a representative clone, as indicated. Such primary sequence data was edited and trimmed of vector sequences and
25 highly repetitive sequences and used to search Genbank databases using the BLAST (Altschul, S.F. et al., 1990, J. Mol. Biol. 215:403-410) program.

Northern analysis: RNA samples were electrophoresed in
30 a denaturing agarose gel containing 1-1.5% agarose (SeaKem[™] LE, FMC BioProducts, Rockland, ME) containing 6.3% formaldehyde. Samples containing 5-20 μ g of total RNA were mixed with denaturing loading solution (72% deionized formamide and bromophenol blue) and heated to 70°C. for 5
35 minutes. Samples were placed on ice and immediately loaded onto gels. Gels were run in 1x MOPS buffer (100mM MOPS, 25mM sodium acetate, 5mM EDTA). After electrophoresis, the gels

were stained with ethidium bromide and visualized with ultraviolet light.

After completion of electrophoresis, gels were soaked in 50mM sodium hydroxide with gentle agitation for approximately 5 30 minutes to lightly cleave RNA. Gels were rinsed twice in water and then neutralized by soaking in 0.1M Tris-HCl (pH 7.5) for approximately 30 minutes. Gels were briefly equilibrated with 20x SSC (3M sodium chloride, 0.3M sodium citrate) and then transferred to nylon membranes such as 10 Hybond™, -N, (Amersham, Inc., Arlington Heights, IL) or Zeta-Probe (Bio-Rad, Inc., Hercules, CA) overnight in 20x SSC. Membranes containing transferred RNA were baked at 80°C. for 2 hours to immobilize the RNA.

DNA fragments to be used as probes were of various sizes 15 and were labeled using a random hexamer labeling technique. Briefly, 25ng of a purified DNA fragment was used to generate each probe. Fragments were added to a 20μl random hexanucleotide labeling reaction (Boehringer Mannheim, Inc., Indianapolis, IN) containing random hexamers and a mix of the 20 nucleotides dCTP, dGTP, and dTTP (at a final concentration of 25μM each). The reaction mix was heat-denatured at 100°C. for 10 minutes and then chilled on ice. 5μl of α-³²P-dATP (50μCi; Amersham, Inc., Arlington Heights, IL) and Klenow DNA polymerase (2 units; Boehringer Mannheim, Inc., Indianapolis, 25 IN) were added. Reactions were incubated at 37° for 30 minutes. Following incubation, 30μl water was added to the labeling reaction and unincorporated nucleotides were removed by passing the reactions through a BioSpin-6™ chromatography column (Bio-Rad, Inc., Hercules, CA). Specific incorporation 30 was determined using a scintillation counter. 1-5 x10⁶ cpm were used per ml hybridization mixture.

Nylon membranes containing immobilized RNA were prehybridized according to manufacturer's instructions. Radiolabelled probes were heat denatured at 70°C. in 50% 35 deionized formamide for 10 minutes and then added to the hybridization mixture (containing 50% formamide, 10% dextran sulfate, 0.1% SDS, 100μg/ml sheared salmon sperm DNA, 5x SSC,

5x Denhardt's solution, 30mM Tris-HCl (pH 8.5), 50mM NaPO₄ (pH 6.5). Hybridizations were carried out at 42°C. overnight. Nylon membranes were then bathed for 2 minutes in a wash solution of 0.2x SSC and 0.1% SDS at room temperature 5 to remove most of the remaining hybridization solution. The membranes were then bathed twice in fresh 42°C. preheated wash solution for 20 minutes. Filters were covered in plastic wrap and exposed to autoradiographic film to visualize results.

10

6.2. RESULTS

A transgenic T cell paradigm (as described, above, in Section 6.1) was utilized to identify genes which are differentially expressed between TH1 and TH2 cells.

15 RNA samples were isolated from TH1 and TH2 cell populations after either secondary or tertiary antigen stimulation. The samples were then analyzed via differential display techniques. FIG. 1 shows amplified fragments obtained from these samples, with the arrow indicating a PCR 20 product, designated band 102, which was judged to represent a cDNA derived from RNA produced by a gene which is expressed at a higher level in TH2 cell subpopulations, relative to TH1 cell subpopulations. The gene corresponding to band 102 is referred to herein as the 102 gene.

25 The amplified band 102 cDNA was recovered, reamplified, subcloned into a cloning vector and sequenced, as described, above, in Section 6.1. The nucleotide sequence (SEQ ID NO:1) of a representative band 102 clone, specifically, clone 102.1, is shown in FIG. 2.

30 A BLAST (Altschul, S.F. et al., 1990, J. Mol. Biol. 215:403-410) database search with this consensus sequence resulted in an alignment with 98% identity to the mouse Granzyme A, or Hanukah factor, gene, (Masson, D. et al., 1986, FEBS Lett. 208:84-88; Masson, D. et al., 1986, EMBO J. 35 5:1595-1600; Gershensfeld, H.K. and Weissman, I.L., 1986, Science 232:854-858), which encodes a trypsin-like serine protease. The human homolog of this gene is also known

(Gershenfeld, H.K. et al., 1988, Proc. Natl. Acad. Sci. USA 85:1184-1188).

To confirm the gene's putative differential regulation, amplified band 102 cDNA was used to probe Northern RNA blots containing RNA samples from TH1 and TH2 cell lines, and from spleen and thymus tissue. FIG. 3 shows the results of one such Northern blot analysis, in which the steady state level of message for 102 gene mRNA are significantly increased in RNA samples derived from stimulated TH2 versus TH1 samples. Further, the positive signals in both thymus and spleen RNA samples supports the indication that the 102 gene product is involved in some aspect of T cell function. Thus, the Northern analysis confirmed the putative differential TH2 regulation which had been suggested by the differential display result.

Therefore, by utilizing the transgenic T cell paradigm described in this Section and in Section 5.1.1.1, above, a TH2 differentially regulated gene, designated here the 102 gene, and corresponding to the mouse Granzyme A/Hanukah factor gene, has been identified, thereby corroborating the usefulness of such paradigms in identifying genes expressed preferentially in T helper cell subpopulations such as TH1 or TH2 cell populations.

Further, while the gene identified here had previously been found to be expressed in natural killer T cells and, further, had been reported to be expressed in a fraction of CD4⁺ cells (Fruth, U. et al., 1988, Eur. J. Imm. 18:773-781; Liu, C.C. et al., 1989, J. Exp. Med. 170:2105-2118), the results described herein represent the first instance in which a TH cell subpopulation role for this gene has been found. Prior to this study, the gene had been reported to be expressed in T cells in a variety of situations, including TH1 cell subpopulation- and TH2 cell subpopulation-related disorders. For example, Granzyme A/Hanukah factor expression has been reported in allograft rejection (Muller, C. et al., 1988, J. Exp. Med. 167:1124-1136) and autoimmune diseases (Ojcius, D.M. and Young, D.E., 1990, Cancer Cells 2:138-145;

Young, L.H.Y. et al., 1992, Am. J. Path. 140:1261-1268), which are TH1 cell subpopulation-related disorders, and also in Leishmania infection susceptible mice (Moll, H. et al., 1991, Inf. and Imm. 59:4701-4705) and in leprosy lesions (Ebnet, K. et al., 1991, Int. Imm. 3:9-19; Cooper, C.L. et al., 1989, J. Exp. Med. 169:1565-1581), which are both TH2 cell subpopulation-related disorders.

The differential TH2-like expression demonstrated here represents, therefore, the first molecular evidence clearly indicating a more primary role for the gene product in TH2 versus TH1 cell subpopulations.

7. EXAMPLE: IDENTIFICATION AND CHARACTERIZATION OF A TH2-SPECIFIC GENE

In the Example presented in this Section, the transgenic T cell paradigm, described, above, in Sections 5.1.1.1 and 6, was utilized to identify a gene which is differentially expressed in TH2 cells. Specifically, this gene is present in TH2 cells while being completely absent from TH1 cells. The gene, which corresponds to a gene known, alternatively, as ST-2, T1 and Fit-1, does not appear to be expressed in any other assayed cell type or tissue, and is demonstrated here for the first time to encode a marker which is, in vivo, completely TH2-specific. The 103 gene encodes a cell surface protein, the potential significance of which is discussed herein.

7.1. MATERIALS AND METHODS

RT-PCR analysis: Quantitative RT-PCR was performed as follows. 1-2 μ g of total RNA, prepared as described, above, in Section 6.1, was reverse transcribed with oligo dT₍₁₂₋₁₈₎ primers and SuperscriptTM RNAase H⁻ reverse transcriptase (Gibco-BRL, Gaithersburg, MD). Briefly, RNA was combined with 1 μ l oligo dT (500 μ g/ml) in a total volume of 11 μ l. The mixture was heated to 70° C for 10 minutes and chilled on ice. After a brief centrifugation, RNA was reverse

transcribed for 1 hour. Aliquots of the first strand cDNA were stored at -20° C. until just prior to use.

Expression levels were determined by PCR amplification of serial dilutions of first strand cDNA. In this procedure, 5 cDNA is serially diluted in water. The dilutions are then batch amplified by PCR using sequence-specific primers. All PCR reactions are amplified under identical conditions. Therefore, the amount of product generated should reflect the amount of sequence template which was initially present. 5-10 10 fold dilutions of cDNA were used and enough dilutions were used such that the amount of product subsequently produced ranged from clearly visible, by UV illumination of ethidium bromide-stained gels, to below detection levels. The method described herein can distinguish 10-fold differences in 15 expression levels.

Primers were designed for the amplification of the sequenced amplified bands, which were chosen using the program OLIGO (National Biosciences, Plymouth, MN). Primer sequences used in this assay were as follows: and 103 sense 20 primer, 5'-TTGCCATAGAGAGACCTC-3' (SEQ ID NO:18); band 103 antisense primer, 5'-TGCTGTCCAATTATACAGG-3' (SEQ ID NO:19); murine gamma actin sense primer, 5'-GAACACGGCATTGTCTAACT-3' (SEQ ID NO:20); murine gamma actin antisense primer, 5'-CCTCATAGATGGGCACTGTGT-3' (SEQ ID NO:21).

25 All quantitative PCR reactions were carried out in a 9600 Perkin-Elmer PCR machine (Perkin-Elmer). Generally, amplification conditions were as follows: 30-40 cycles consisting of a 95° C denaturation for 30 seconds, 50-60° C annealing for 30 seconds, and 72° C extension for 1 minute. 30 Following cycling, reactions were extended for 10 minutes at 72° C.

RNAse Protection Assays: RNAse protection assays were performed according to manufacturer's instructions, using a 35 kit purchased from Ambion, Inc. RNA probes derived from GenBank Accession No. Y07519 were utilized in the RNAse protection assays. These probes were also generated

according to manufacturer's instructions, using a kit purchased from Ambion, Inc. The sequence of these RNA probes corresponds to the 5' end of the gene, and includes both coding and 5' untranslated sequences.

5

Anti CD-3 stimulation: Conditions were as described, below, in Section 8.1.

Other procedures: All other cell sample collection, RNA
10 isolation, differential display, sequence analysis, and Northern procedures performed in the experiments described in this Example were as described, above, in Section 6.1.

7.2. RESULTS

15 A differential display analysis of RNA isolated from TH1 and TH2 cell samples obtained from a transgenic T cell paradigm study as described, above, in Section 6.1. Specifically, TH cells were obtained from transgenic mice harboring a T cell receptor recognizing ovalbumin (Murphy et
20 al., 1990, Science 250:1720) were stimulated three times, and RNA was obtained from TH1 and TH2 cells. Differential display analysis of the RNA samples resulted in the identification of a TH2 differentially expressed band, designated and referred to herein as band 103. The gene
25 corresponding to band 103 is referred to herein as the 103 gene.

103 gene cDNA was isolated, amplified and subcloned, and nucleotide sequence (SEQ ID NO:2) was obtained, as shown in FIG. 4A. A database search revealed that the nucleotide
30 sequence of band 103 resulted in an alignment with 98% identity to the mouse form of a gene known, alternatively, as the ST-2, T1 or Fit-1 gene (Klemenz, R. et al., 1989, Proc. Natl. Acad. Sci. USA 86:5708-5712; Tominaga, S., 1989, FEBS Lett. 258:301-301; Werenskiold, A.K. et al., 1989, Mol. Cell.
35 Biol. 9:5207-5214; Werenskiold, A.K., 1992, Eur. J. Biochem. 204:1041-1047; Yanagisawa, K. et al., 1993, FEBS Lett. 318:83-87; Bergers, G. et al., 1994, EMBO J. 13:1176-1188).

The 103 gene encodes, possibly via alternatively spliced transcripts, transmembrane and soluble forms of proteins which belong to the immunoglobulin superfamily. The soluble form of the protein shows a high level of similarity to the
5 extracellular portion of the mouse interleukin-1 receptor type 1 (IL-1R1) and interleukin-1 receptor type 2 (IL-1R2; which lacks a cytoplasmic domain), while the transmembrane portion (termed ST2L) bears a high resemblance to the entire IL-1R1 sequence and to the extracellular IL-1R2 sequences.
10 Further, the 103 gene appears to be tightly linked to the interleukin 1 receptor-type 1 locus (McMahan, C.J. et al., 1991, EMBO J. 10:2821-2832; Tominaga, S. et al., 1991, Biochem. Biophys. Acta. 1090:1-8). Additionally, the human 103 gene homolog has also been reported (Tominaga, S. et al.,
15 1992, Biochem. Biophys. Acta. 1171:215-218). FIG. 4B illustrates the 103 gene transmembrane and soluble forms of protein, and shows their relationship to the IL-1R1 protein sequence.

A quantitative RT-PCR analysis (FIG. 5) of RNA obtained
20 from cells of a TH1 and TH2 cells, generated as described above, 24 hours after tertiary antigen stimulation not only confirmed the putative TH2 differential expression of the gene, but, revealed that the expression of the 103 gene appears to be TH2 specific, i.e., the sensitive RT-PCR study
25 detected no 103 gene message in the TH1 RNA sample.

The TH2 specificity of the 103 gene was further confirmed by a Northern analysis of several representative TH cell lines. Specifically, three TH2 clones (CDC25, D10.G4, DAX) and three TH1 clones (AE7.A, Dorris, D1.1) were utilized
30 and RNA samples were isolated from either unstimulated cells or from cells which had been stimulated for 6 hours with plate-bound anti-CD3 antibody. The samples were probed with band 103 sequences, as shown in FIG. 6. While 103 gene RNA is present in RNA obtained from both unstimulated and
35 stimulated cells of each of the TH2 cell lines, 103 gene RNA is completely absent from all of the samples obtained from either stimulated or unstimulated TH1 cells. As the RT-PCR

analysis described above first demonstrated, the 103 gene appears to be TH2 specific, with no detectable TH1-derived signal being present.

The data presented in FIG. 7 represent an additional Northern analysis in which 103 gene expression was assayed in TH cell clones (lanes 1-5) and in murine tissues (lanes 6-10). In addition to corroborating the expression of 103 gene RNA in both stimulated and unstimulated TH2 cells, the data presented here demonstrate that 103 gene expression appears to be negative in each of the tissues (i.e., brain, heart, lung, spleen, and liver) tested.

FIG. 8 illustrates an RNase protection assay which demonstrates two points regarding 103 gene regulation. First, this analysis of TH cell clones confirms the TH2-specific results described, above. Specifically, the results of this study demonstrate by RNase protection, that 103 gene mRNA is absent from the TH1 clone AE7, but is present in the TH2 clone D10.G4.

Second, RNase protection revealed that alternate forms of 103 gene transcripts are produced upon stimulation of TH2 clones. Specifically, within 6 hours of anti-CD3 stimulation, two additional forms of 103 gene transcript appear in TH2 clones. These additional 103 gene transcript forms represent, one, a transcript encoding a shortened, secreted, soluble form of the band 103 gene product, and, two, a smaller, termed mini, transcript which encodes a yet shorter form of the gene product. Thus, it appears that, while the 103 gene transcript encoding the transmembrane gene product is expressed in both unstimulated and stimulated TH2 cells, the two shorter forms of transcript are expressed in a TH2-specific inducible manner. Further, while the 103 gene transcript encoding the transmembrane product are expressed in both stimulated and unstimulated TH2 cells, the level of this transcript present in stimulated is lower, i.e., is downregulated. Thus, the lower level of transmembrane product and higher level of secreted 103 gene product can act

synergistically to dampen some stimulation-induced signal transduction event.

Additionally, it should be noted that the results presented herein represent the first time the mini form of 5 103 gene transcript, which can encode a shorter version of the soluble form of 103 gene product, has been observed.

To summarize, while 103 gene expression in T helper cell lines had previously been reported (Tominaga, S. et al., 1992, Biochem. Biophys. Acta. 1171:215-218), the TH 10 paradigm/differential display techniques utilized here have demonstrated, for the first time, that the 103 gene encodes a TH2 cell subpopulation-specific surface marker. In fact, the results described in this Example demonstrate that the first identification of any in vivo TH cell subpopulation-specific 15 cellular marker.

Given its status as both a TH2 cell subpopulation-specific marker and cell surface protein, the full length 103 gene product can be utilized in a variety of methods to modulate TH cell subpopulation-related disorders and/or to 20 identify compounds which exhibit such modulatory capability. The truncated forms of the 103 gene products can, additionally, be used as part of these methods. Modulatory methods are described, above, in Section 5.9, while strategies for the identification of modulatory compounds are 25 described, above, in Section 5.8.

8. EXAMPLE: IDENTIFICATION OF NOVEL TH CELL SUBPOPULATION DIFFERENTIALLY EXPRESSED GENES

In the Example presented in this Section, novel gene 30 sequences representing genes which are differentially expressed in TH cell subpopulations and/or during the differentiation of such subpopulations are described.

8.1. MATERIALS AND METHODS

35 T cell clone paradigm: T cell clone paradigm searches were conducted as described, above, in Section 5.1.1.1. Specifically, the TH cell clone paradigms used three

different clones: D10.G4 (TH2), AE7 (TH1) and D1.1 (TH1). Prior to stimulation, cell cultures were enriched for live cells by centrifugation through a Ficoll gradient. Recovered cells were counted and their viability was examined using
5 trypan blue exclusion. Cells were replated into either T25 or T75 flasks at approximately 5×10^6 cells in 5 mls or 1.5×10^6 cells in 10 mls of culture medium, respectively.

Coating was performed, generally, according to Current Protocols in Immunology, 1992, Coligan, J.E. et al., John
10 Wiley & Sons, NY, pp 3.12.4-3.12.6). Specifically, prior to plating, the flasks were coated with anti-CD3- ϵ antibodies (hybridoma supernatant from the 145-C11 hybridoma; Parmingen, Inc., San Diego CA). For coating, antibodies were resuspended in PBS at 1-2 $\mu\text{g/ml}$ at a volume sufficient to coat the bottom
15 of the flasks. Coating solution was incubated on the flasks for at least one hour at 37° C.

After incubation, the antibody coating solution was removed by aspiration and cells were immediately added. Flasks were placed in a 37° C incubator for 6 hours. Cells
20 were harvested by, for example, removal of supernatant from the culture, followed by direct lysing of cells by addition of RNazol™ solution. cDNA was produced as described below.

cDNA isolation: RNA was harvested from cells using techniques described, above, in Section 6.1. mRNA was
25 purified directly, using a QuickPrep™ mRNA Purification Kit (Pharmacia) according to manufacturer's instructions.

The TH1 cDNA library was constructed using a Gibco BRL SuperScript™ Lambda System Kit, according to manufacturer's instructions. Briefly, 4.5 μg of purified mRNA was used as
30 starting material for the synthesis of poly A-primed first strand cDNA containing a Not-1 cloning site. Second strand cDNA synthesis was performed with RNase H treatment followed by random priming. Sal-1 adaptors were ligated to the 5' end of the resulting double-stranded cDNA. The ligated cDNA was
35 digested with Not-1 and size fractionated. Fractions containing cDNAs within the size range of 0.5 to 8.0 kb in length were cloned into Sal-1/Not-1 λ ZipLox™ arms.

Recombinant phage was then packaged using the Stratagene Gigapack™ II Packaging Extracts Kit, according to manufacturer's instructions. E. coli strain Y 1090(ZL)™ (Gibco BRL) cells were transformed with packaged recombinant phage and plated at a density of 50,000 pfu per 150mm dish. Plaques were screened by hybridization to a radiolabelled probe generated from a subcloned band 200 cDNA fragment. Excision of cDNA inserts from lambda clones and introduction of the recombinant plasmid DNA into E. coli DH10B(ZIP)™ (Gibco BRL) was performed according to manufacturer's instructions.

For isolation of 200 gene cDNAs, the cDNA library was screened with a probe generated by labeling the entire sequence of the band 200 subclone O, which was constructed using amplified DNA obtained from the differential display analysis. The band 200 sequence was excised from the pCRII Cloning Vector™ (Invitrogen) by digestion with EcoRI. Approximately 1/100,000 cDNA library plaques were scored as positive when screened with this probe. Several clones, including 200-P and 200AF, were chosen for further study.

The cDNA library described above was also used to isolate 54 gene cDNA clones. For screening, the entire excised band 54 insert was used as a probe.

Other procedures: All transgenic T cell manipulations, cell sample collection, additional RNA isolation, differential display, sequence analysis, and Northern procedures performed in the experiments described in this Example were as described, above, in Section 6.1.

30 8.2. RESULTS

Transgenic T cell paradigm and T cell clone paradigm searches were conducted to identify gene sequences which represent genes differentially expressed within and/or among TH cell subpopulations and/or during the differentiation of such subpopulations. Described herein are several novel genes which have been identified via these paradigm searches. Specifically, the genes described herein have been designated

the 10, 54, 57, 105, 106, 161 and 200 genes. A summary of the differential expression characteristics of the novel gene sequences described herein is presented in Table 1, above.

The band 10 and 57 have been identified as TH inducible gene sequences. That is, the expression of such genes in unstimulated TH cells is either undetectable or is detectable at extremely low levels, but is upregulated in both stimulated TH1 and TH2 cells. In fact, the 10 gene expression is detectable as early as 6 hours post stimulation. Thus, such gene products can be involved in the activation of TH cells and/or can be involved in the maintenance of mature TH cell function, in a non-TH cell subpopulation-specific manner.

FIG. 9 depicts the nucleotide sequence (SEQ ID NO:3) of the 10 gene coding region and the derived amino acid sequence of the 10 gene product (SEQ ID NO:10). While database searches reveal that the 10 gene sequence is novel, that is, has not previously been reported in the databases, an analysis of the portion of the 10 gene corresponding to the band 10 nucleotide sequence (the underlined portion of the nucleotide sequence of FIG. 9) shows, as depicted in FIG. 10A-C, a high similarity to a specific class of known gene products. Specifically, as the hydrophilicity plots of FIG. 10A-C show, the 10 gene product appears to encode a protein having a seven transmembrane domain sequence motif.

Interestingly, the gene products belonging to this class of protein tend to represent G protein-coupled receptor molecules. (See, e.g., Larhammar, D. et al., 1992, J. Biol. Chem. 267: 10935-10938; Law, S.F. et al., 1991, J. Biol. Chem. 266: 17885-17997.) Thus, the TH inducible expression of the 10 gene coupled with the predicted protein structure of its gene product, suggests that the 10 gene product is involved in a signal transduction event important to the differentiation of mature TH cells.

Additionally, as the map shown in FIG. 11 indicates, the chromosomal location of the murine 10 gene has been identified. The 10 gene locus is located on Chromosome 12,

is closely linked to a class of genes encoding T cell autoantigens, and additionally, maps near the Ig heavy chain gene locus.

The nucleotide sequence (SEQ ID NO:4) of a representative band 57 clone is depicted in FIG. 12. The gene corresponding to band 57 is the 57 gene. The 57 gene appears to be a novel gene sequence in that it does not appear within the published databases. No homology to known peptide domains has, thus far, been identified.

As shown in Table 1, above, the genes 105, 106 and 200 are each expressed at a higher level within the TH1 cell subpopulation, as revealed by the TH1 differential appearance of amplified bands 105, 106 and 200. Nucleotide sequences contained within bands 105 and 106 are depicted in FIGS. 13 (SEQ ID NO:5) and 14 (SEQ ID NO:6), respectively. As discussed below, the sequence of the murine 200 gene is depicted in FIG. 17 (SEQ ID NO:8). None of these sequences appear within published databases. Given the TH1-specific expression pattern each of these sequences exhibits, the genes and their gene products can potentially be used as treatments for TH1-related disorders, as diagnostics for such disorders, and/or as part of methods for the identification of compounds capable of ameliorating TH1-related disorders.

The 161 gene appears to be TH cell subset specific. That is, 161 gene expression has been observed in either TH1 cells or in TH2 cells, but its expression has never been observed, simultaneously, in both TH1 and TH2 cell subpopulations. The details of the 161 gene differential expression pattern are currently being elucidated. It is possible that 161 gene expression is indicative of the presence of yet another TH cell subpopulation, in addition to TH1, TH2 and TH0 cell subpopulations.

FIG. 15 presents the band 161 nucleotide sequence. While the 161 gene appears to be a novel sequence, it bears a distinct level of similarity to a set of gene sequences (SEQ ID NOS:13-17) in published databases, as shown in FIG. 16.

Interestingly, the genes within this group each contain alpha-interferon responsive promoters.

Band 200 was utilized as a probe to identify and isolate murine 200 gene cDNA clones, including clones designated 5 200-P, 200-AF and 200-O, which have been deposited with the NRRL, as summarized in Section 10, below. The cDNA clones were characterized, yielding the full length nucleotide sequence (SEQ ID NO:8) of the murine 200 gene coding region, as shown in FIG. 17. FIG. 17 also depicts the murine 200 10 gene product derived amino acid sequence (SEQ ID NO:10). Database searches reveal that the 200 gene product is a novel receptor which contains an extracellular Ig domain, thus placing it within the Ig receptor superfamily. The cloning and characterization of the 200 gene human homolog is 15 described in the Example presented in Section 9, below.

The results of a murine 200 gene mRNA Northern blot analysis are shown in FIG. 18. The data depicted in FIG. 18 demonstrates, first, that the 200 gene produces a transcript of approximately 1.2 kb in length, and, second, illustrates 20 the TH1 specificity of 200 gene expression

For the study, three TH1 clones (D1.1, Dorris, AE7) and three TH2 clones (D10G.4, DAX, CDC25) were utilized, and RNA samples were isolated from either unstimulated cells (-) or cells which had been stimulated for 6 hours with plate-bound 25 anti-CD3 antibody (+). The samples were probed with 200 gene sequences, and, as shown in FIG. 18, RNA from both stimulated and unstimulated TH1 cells contained 200 gene mRNA, while none of the samples obtained from TH2 cells contained 200 gene mRNA. It should also be noted that 200 gene expression 30 was higher in each of the stimulated TH1 cells relative to the corresponding unstimulated TH1 cells.

As shown in Table 1, above, the 54 gene is expressed in a TH1-restricted manner. The 54 gene was identified via T cell paradigm searches in which the expression pattern of a 35 TH1 cell clone, AE7, was compared to that of a TH2 cell clone, D10.G4. The initial differential expression analysis

was performed using differential display techniques, as described, above, in Section 6.1.

The TH1-restricted pattern of the 54 gene expression was corroborated through Northern analysis of RNA isolated from 5 TH1 cell lines (AE7, D1.1, Dorris) and TH2 cell lines (D10.G4, DAX, CDC25), as shown in FIG. 19. The TH1/TH2 Northern blot data depicted in FIG. 19 additionally illustrates 54 gene expression within cell clones either stimulated or unstimulated with anti-CD3 antibodies, and 10 demonstrates that 54 gene expression goes down within stimulated TH1 cells.

To further characterize the 54 gene expression, a detailed time course study was conducted using RNA isolated from AE7 clones. Specifically, RNA was isolated from 15 unstimulated AE7 clones as well as from AE7 clones which had been stimulated with anti-CD3 antibodies for varying lengths of time, as noted in FIG. 20. As illustrated in FIG. 20, 54 gene expression decreased slightly by 2-6 hours after stimulation and had not again achieved pre-stimulation levels 20 within 48 hours after stimulation.

A 54 gene expression analysis of cell lines representing a variety of T cells, B cells and monocytic/macrophage cell lines was performed which failed to detect 54 gene expression in non-TH1 cells, demonstrating that 54 gene expression is 25 highly restricted to TH1-like cells. A Northern analysis of 54 gene expression within tissues (FIG. 21), also demonstrated an expression profile consistent with that of a TH1 cell-restricted expression profile. Namely, as shown in FIG. 21, most organs failed to express the 54 gene, while the 30 highest level of 54 gene expression was seen in lymph node tissue, and lowest detectable level of expression was seen in spleen, testis and uterus.

Band 54 nucleotide sequence, which had been obtained from the amplified cDNA produced in the initial differential 35 display analysis in which the 54 gene was identified, was used to isolate seven cDNA clones, designated 54A-G. Each of

the clones were of similar size. The 54-C cDNA has been deposited with the NRRL within the E. coli clone, 54-C.

FIG. 22 shows the entire 54 gene coding sequence (SEQ ID NO:11). The derived amino acid sequence of the 54 gene product is also shown in FIG. 22 (SEQ ID NO:12). Based on database homology searches, the 54 gene appears to encode a novel cysteine protease. Cysteine proteases are enzymes which contribute to intracellular protein degradation and appear to play a role in tissue degradation. It is possible, therefore, that the inhibition of 54 gene expression and/or 54 gene product activity in immune disorders involving TH1-like cells may serve to minimize any tissue damage.

Specifically, the 54 gene sequence exhibits the three thiol protease domains typical of active cysteine protease enzymes. These domains include a CYS domain at approximately amino acid residue 145 to 156 (active site: C, position 151), a HIS domain at approximately amino acid residue 287 to 297 (active site: H, position 289), and an ASN domain at approximately amino acid residue 321 to 340 (active site N, position 326). Interestingly, the typical CYS domain is broken by a K residue at position 149 (this position is usually G or E), perhaps indicating that the 54 gene product cysteine protease is very substrate-specific. Additionally, amino acid sequence analysis indicates probable disulfide bonds between cysteines at 148 and 189, 182 and 224 and 282 and 347. Further, FIG. 23 depicts the 54 gene product amino acid sequence and points out some of its potential cysteine protease-like features. For example, the 54 gene product has an amino terminal end which resembles a cysteine protease preproenzyme region, which is cleaved away upon formation of the active cysteine protease. The boxed region, from amino acid residue 56 to 75 represents an "ERFNIN" region which has previously been noted as a feature of several cysteine proteases (Ishidoh, K. et al., 1987) FEBS Lett. 226:33-37). The circled amino acid residues within the boxed region represent conserved amino acid residues. The individual boxed amino acid residues represent residues that, based on

homology, are thought to lie within the active site of the enzyme.

9. EXAMPLE: IDENTIFICATION AND CHARACTERIZATION OF HUMAN 200 GENE

In the Example presented herein, the cloning, identification and characterization of the human 200 gene, corresponding to the human homolog of the murine 200 gene, is described.

9.1. MATERIALS AND METHODS

Murine 200 gene probe: An approximately 800 bp EcoRI insert containing about 90% of the murine 200 gene cDNA (feht200). ORF was gel purified, ^{32}P labelled, and used to probe the $\lambda\text{gt}11$ human lymphocyte cDNA library described below.

Human 200 gene probe:

The approximately 500 bp insert of the human 200 gene feht200a cDNA clone was ^{32}P labelled and used to probe the human fetal spleen cDNA library described below.

Screening procedures: Approximately 10^6 plaques of a $\lambda\text{gt}11$ human lymphocyte cDNA library (Catalog No. HL 1031B; Clontech) were screened with murine 200 gene probe described above in duplicate. The filters were hybridized with probe overnight at 65°C in Church's buffer (7% SDS, 250mM NaHPO_4 , $2\mu\text{M}$ EDTA, 1%BSA). The next day, filters were washed in 2XSSC/1% SDS for 30 min at 50°C . The filters were then exposed to Kodak film at -80°C . Positive plaques were rescreened under the same conditions.

A human fetal spleen cDNA library constructed using the Stratagene Uni-Zap cloning System was screened using the human feht200a gene probe described above. Approximately 10^6 plaques were hybridized in duplicate at 65°C in Church's buffer overnight. The filters were then washed for 30 min at 65°C in 0.1XSSC, 0.1% SDS and exposed to film. Positives were confirmed by secondary screening under the same conditions.

Subcloning/Sequencing procedures:

DNA from the positive clones obtained from the λ gt11 cDNA library was generated by a plate lysis method. The purified DNA was digested to obtain cDNA inserts which were 5 subcloned into the pBluescript plasmid (Stratagene).

Positive clones obtained from the human fetal spleen cDNA library were excised with ExAssist helper phage, XL1-Blue cells and SOLR cells as described by Stratagene.

Excision products were then plated out on LB/Amp plates and 10 incubated at 37°C overnight. White colonies were picked and DNA prepared for sequencing.

DNA sequencing was performed according to standard techniques.

Northern blot analysis of human gene 200 expression:

15 Northern blots were carried out as described in Section 6.1, above. 15 μ g of total RNA from a variety of human organs were analyzed (Clontech, CA). The 32 P labelled probe utilized was the feht200a clone, described above, which contains the 5' ORF of human gene 200.

20

9.2 RESULTS

The full length sequence of the human 200 gene was successfully cloned and characterized, as described herein.

In order to clone human 200 gene, an 800 bp EcoRI insert 25 containing approximately 90% of the murine 200 gene cDNA (femt200) ORF was gel purified, 32 P labelled, and used to probe a λ gt11 human lymphocyte cDNA library. Approximately 10^6 plaques were screened in duplicate, as described in Section 9.1, above. One positive plaque was obtained and 30 rescreened under the same conditions. Once pure, this clone was used to generate lambda DNA by a plate lysis method, and the lambda DNA was digested to obtain a 500 bp insert (feht 200a) which, upon sequencing, was found to be a human homologue of the murine 200 gene.

35 To obtain a clone encoding the entire ORF of the human 200 gene, a human 200 gene probe was used to screen a human fetal spleen cDNA library, as described in Section 9.1.,

above. Three positive clones were obtained, two of which were positive upon secondary screening under the same conditions. The two positive clones were subcloned and their cDNA inserts were sequenced. These two clones labelled
5 feht200b and feht200c were approximately 1.56 kb and 2.0 kb in length, respectively with feht200c containing the entire coding sequence. Clone feht200c was deposited with the ATCC, as described, below, in Section 12.

The nucleotide sequence containing the complete human
10 200 gene open reading frame is depicted in FIG. 24 (SEQ ID NO: 37). The derived amino acid sequence of the human 200 gene product is also depicted in FIG. 24 (SEQ ID NO: 24).

The 301 amino acid residue sequence of the human 200 gene product reveals that it is a cell surface receptor
15 exhibiting distinct domains, including a signal sequence from amino acid residue 1 to approximately 20, an extracellular domain from approximately amino acid residue 21 to 200, a transmembrane domain from approximately amino acid residue 201-224 and a cytoplasmic domain from approximately amino
20 acid 225 to 301. The extracellular domain contains an Ig type variable set domain from approximately amino acid residue 30 to approximately amino acid residue 128, thus placing the 200 gene product within the Ig receptor superfamily.

25 A Northern analysis of the tissue distribution of 200 gene transcripts was performed. 15 μ g RNA from brain, kidney, liver, lung, muscle, prostate, spleen, thymus and trachea were isolated and analyzed for human 200 gene expression. This analysis revealed human 200 gene
30 transcripts of approximately 2.2 kb, in tissues including brain, lung, trachea, spleen and thymus.

In summary, the human 200 gene, corresponding to the human analog of the murine 200 gene, has been successfully cloned and characterized, as described herein. As revealed
35 by its amino acid sequence, the human 200 gene product is a receptor of the Ig superfamily class of molecules.

10. EXAMPLE: CONSTRUCTION AND EXPRESSION OF IgG1 FUSION PROTEINS

Described in this Example is the construction and expression of IgG1 fusion proteins. Specifically, the construction of human and murine 200 gene and 103 gene IgG1 fusion proteins are discussed.

10.1. MATERIALS AND METHODS

Recombinant plasmids encoding IgG1 fusion proteins:

10 Generation of the vector encoding murine 200 gene-hIgG1 fusion protein: The fragment encoding the signal sequence and extracellular domain of murine 200 gene was amplified from a cDNA clone containing the ORF of murine 200 gene using the following oligonucleotides:

15 Forward oligo: 5'-AAA-TTT-ATT-CTC-GAG-GAC-CCA-CGC-GTC-CGG-ATT-TCC-C-3' (SEQ ID NO: 25);

Reverse oligo: 5'-TTA-ATT-TGG-ATC-CCC-AGT-TCT-GAT-CGT-TTC-TCC-AGA-GTC-3' (SEQ ID NO: 26).

20 The oligonucleotide primers also introduce XhoI and BamHI restriction sites at the 5' and 3' ends of the PCR products, respectively, to facilitate the subsequent insertion into IgG1 expression vectors (pCD5-CD44-IgG1; see Aruffo, A. et al., 1991, Cell 61:1303-1313). The pCD5-CD44-IgG1 vector encodes a protein containing a CD5 signal sequence, a CD44 extracellular domain and a human IgG1 heavy chain Fc region. For construction of the murine 200 gene-hIgG1 fusion protein vector, the CD5 and CD44 portions of pCD5-CD44-IgG1 were replaced with sequences encoding murine 200 gene product signal sequence and extracellular domain.

30 The PCR reactions consisted of 25 cycles amplification at an annealing temperature of 60°C. Vent™ thermostable DNA polymerase (New England BioLabs, Inc.; Beverly, Massachusetts) was used in the amplification. The PCR product (approximately 600 bp) was digested with XhoI and BamHI and inserted into pCD5-CD44-IgG1 previously digested

with XhoI and BamHI to remove the sequences encoding the CD5 signal sequence and the CD44 ectodomain.

Generation of the vector encoding human 200 gene-hIgG1 fusion protein: The fragment encoding the signal sequence and extracellular domain of human 200 gene is amplified from a cDNA clone containing the ORF of human 200 gene using the following oligonucleotides:

Forward oligo: 5'-AAA-TTT-ATT-CTC-GAG-CGC-TAA-CAG-AGG-TGT-CC-
10 3' (SEQ ID NO: 27);

Reverse oligo: 5'-TTA-ATT-TGG-ATC-CCC-TCT-GAT-GGT-TGC-TCC-
AGA-GTC-CCG-3' (SEQ ID NO: 28).

The amplification and pCD5-CD44-IgG1 subcloning procedures
15 are as described, above, for the murine 200 gene-hIgG1 fusion protein.

Generation of the vector encoding the murine 103 gene-hIg G1 fusion protein: The construction of a vector encoding a soluble Ig-fusion protein (size: approximately 60 kD)
20 containing a murine 103 gene product extracellular domain (but lacking the 103 gene product signal sequence) was constructed as described here. The CD44 portion of the pCD5-CD44-IgG1 vector (described above) was replaced with a nucleotide sequence encoding the 103 gene product
25 extracellular domain. The 103 gene product extracellular domain sequence of the Ig-fusion protein consisted of 103 gene product amino acid residues 27-342 (i.e., the 103 gene product portion ending with amino acid sequence Ile-Val-Ala-Gly-Cys-Ser).

30 The fragment encoding the 103 gene product extracellular domain was amplified by PCR using synthetic oligonucleotides complementary to the sequences flanking the 103 gene region that would produce the 103 gene product containing amino acid residues 27-342. The oligonucleotides were designed to allow
35 creation of a KpnI site at the 5' end and a BamHI site at the 3' end of each amplified 103 gene fragment to facilitate subsequent insertion into pCD5-CD44-IgG1.

The 5' oligonucleotide was as follows:

5'-CCGCGGGTACCAGTAAATCGTCCTGGGGTGG-3' (SEQ ID NO: 29).

The 3' oligonucleotide was as follows: 5'-

AAATAAGGATCCCTACATCCAGCAACTATGTAGTA-3' (SEQ ID NO: 30).

- 5 PCR reaction conditions consisted of 15 cycles of 30 seconds at 95°C, 30 seconds at 60°C, and 30 seconds at 72°C, using Vent DNA polymerase (New England Biolabs, Beverly, MA) and 103L gene as template.

- 103 PCR products were digested with KpnI and BamHI, and
10 ligated to KpnI-BamHI sites of CD5-IgG1 vector, thus replacing the CD44 sequences with the 103 gene sequences.

- The resulting plasmid, encoding a fusion protein containing CD5-signal sequence, murine 103-extracellular domain and human-IgG1 heavy chain Fc region, was transfected
15 into COS cells using LipofectAMINE™ (GIBCOBRL, MD) following manufacturer's suggest. 0.18µg plasmid DNA and 140µl LipofectAMINE™ were used for transfection of the cells of a 150mm plate. Twenty-four hours after transfection, medium was replaced with 10% Ultra-low IgG Fetal Bovine Serum
20 (GIBCOBRL, MD)/DMEM(BioWHITTAKER, Maryland), and the transfected cells were allowed to grow for 4-5 days continuously. Supernatants were then harvested, centrifuged to remove nonadherent cells and debris, and stored at -20°C.

- For purification, 1ml of supernatant was precipitated
25 overnight with 10µl of IPA-300 Immobilized rProteinA (Repligen, MA) at 4°C. The next day, beads were collected by centrifugation and washed three times with 10 volumes of PBS. For analysis, the beads were suspended in 20µl of 2 X Laemmli Sample Buffer (BIO-RAD, CA) and boiled at 100°C for 10 min.
30 The boiled sample was spun briefly and loaded onto a 10% SDS-PAGE gel (JILEinc. CT).

- Metabolic labelling of recombinant fusion proteins: 36 hours after transient transfection of COS-7 cultures, cells were rinsed with replacement growth medium [DMEM methionine
35 and cysteine depleted (ICN, Inc., CA)]. After rinsing, 150 µCi/ml medium of a mixture of ³⁵S-cysteine and ³⁵S-methionine

(Express $^{35}\text{S}^{35}\text{S}^{\text{TM}}$, Dupont, MA) was added to the replacement medium and the cells were cultured overnight.

Analysis of recombinant proteins by SDS PAGE:

hIgG1 fusion proteins were generated by LipfectAMINETM (Gibco, Inc., MD)-mediated transient transfection of COS-7 cells according to manufacturer's suggestion for 200 gene-hIgG1 fusion proteins, 1 ml of day 5 supernatant was mixed with 20 μ l of Protein A Trisacryl bead (Pierce, Inc., IL) in the presence of 20mM HEPES (pH 7.0) overnight at 4°C with constant agitation. Beads were then washed 3X with PBS prior to the addition to loading buffer. Beads were mixed with either reducing or non-reducing loading buffers (described in, Molecular Cloning, Sambrook, Fritsch, and Maniatis, 2nd edition, 1989, with the exception that DTT was replaced with 2.5% β -mercaptoethanol).

10.2. RESULTS

The construction and expression of recombinant IgG fusion proteins is described herein. Specifically, 200 gene product-IgG1 and 103 gene product-IgG1 fusion proteins are described. The murine and human 200 gene product-IgG1 fusion protein contains a 200 gene product signal sequence and extracellular domain fusion to a human IgG1 heavy chain Fc region. The 103 gene product-IgG1 fusion protein contains a CD5 signal sequence and 103 gene product extracellular domain fused to a human IgG1 heavy chain Fc region.

200 gene-hIgG1 fusion proteins were produced by transient transfection of COS-7 cells, as described in Section 10.1, above. Protein A immunoprecipitation of the COS-7 supernatants and their analysis by SDS-PAGE demonstrated, first, that the correct IgG-1 peptide was being produced as part of the fusion (as evidenced by the fusion's protein A immunoprecipitation) and, second, demonstrated substantial expression of the 200 gene-IgG1 fusion protein at a concentration approximately 1 μ g per ml of culture supernatant. Further, when the immunoprecipitated supernatants are analyzed and compared under reducing and

non-reducing conditions, it is clear that the 200 gene-IgG1 fusion protein undergoes oligomerization, as expected, given the human IgG1 heavy chain peptide sequence present in the fusion protein. Further, the size (i.e., larger than
5 expected from the amino acid sequence alone) and appearance of the fusion proteins as they migrate through the gels (i.e., diffuse, rather than tight bands) indicate that, as expected, the fusion proteins have been glycosylated.

10 11. EXAMPLE: PRODUCTION AND CHARACTERIZATION OF TRANSGENIC ANIMALS

Described herein is the production and characterization of transgenic mice overexpressing either murine 200 gene product or murine 103 gene product.

15

11.1. MATERIALS AND METHODS

Construction of 200 gene transgenic clone:

A PCR product of the entire 200 gene sequence was used to replace the IL-10 gene in the pCIL-10 plasmid, whose
20 construction is described below.

The pCIL-10 plasmid contained a 5.5 kb *Bam*HI-*Xba*I genomic fragment, within which human CD2 enhancer was included (Greaves et al., 1989, Cell 56(6):979-86). A 0.5 kb
25 *Xba*I-*Sma*I fragment containing human immunoglobulin heavy chain promoter, P_{μ} (Danner and Leder, Proc. Natl. Acad. Sci. USA, 1985, 82:8658-8662), was ligated to the 3'-end of the CD2 fragment. Following the P_{μ} fragment was a *Xba*I (blunt-ended)-*Bam*HI fragment containing the IL-10 coding sequence, to which was ligated the 2.1 kb *Bam*HI-*Eco*RI genomic fragment
30 of human growth hormone (Base 5164 to 7317 of HUMGHCSA (GenBank)) at the 3'-end of the construct.

A 0.8 kb PCR product of the entire murine 200 gene coding sequence was obtained through 25 cycle-reaction using the murine 200 gene cDNA 200-AF as template and
35 oligonucleotides primers with compatible restriction sites *Spe*I at the 5'-end and *Bam*HI at 3'-end. The 5'-oligo utilized was 5'-GCG CAA TTG ACT AGT GAC CCA CGC GTC CGG ATT

TC-3' (SEQ ID NO: 31) and the 3'-oligo, 5'-GAC GCG GAT CCT CAG GAT GGC TGC TGG CTG-3' (SEQ ID NO: 32). After heat denaturation at 95°C for 2 minutes, 3-step cycling was performed for 30 seconds at 95°C, 30 seconds at 60°C, 60
5 seconds at 72°C by Vent™ DNA polymerase (New England Biolabs, MA). A final step for five minutes, at 72°C, was performed for end-polishing. The PCR product was digested by SpeI and BamHI (New England Biolabs, Beverly, MA) and ligated to the fragment of pCIL-10 after removal of SpeI to BamHI of IL-10
10 gene. MaxEfficient *E. coli* DH5α competent cells (GIBCO BRL, MD) were used for transformation following manufacturer's suggestion. Transformants were grown in LB broth containing 0.1 µg/ml ampicillin and the DNA were extracted by Qiagen Plasmid Maxi Kit (Qiagen, CA). Restriction analysis was
15 performed for confirmation, and the final construct was sequenced to eliminate any possible PCR introduced mutations. A plasmid designated p200Tr3 was selected from production of transgenic mice.

This final construct contained an approximately 5.5 kb
20 genomic fragment containing the human CD2 enhancer joined to a 0.5 kb fragment of the human IgM promoter immediately upstream of the murine 200 gene coding sequence. A region containing the 3' untranslated sequence of the human growth hormone gene was positioned immediately downstream of the
25 murine 200 gene ORF and contained a polyA splice site.

Construction of 103 gene transgenic clone:

A PCR product of the entire 103 gene sequence was used to replace the IL-10 gene in the pCIL-10 plasmid. The pCIL-10 plasmid was as described in this Section, above. A
30 PCR product of the entire murine long form of the 103 gene (Yanagisawa, K. et al., 1993, FEBS 318:83-87) coding sequence was obtained through 35 cycle-reaction using first-strand cDNA from a mouse TH2-type cell line, D10G4 (ATCC, MD), as template. Total RNA was extracted from the cell line by
35 RNazole™ (TEL-TEST, Inc., TX). Seven micrograms RNA were used in a 20 µl first-strand cDNA synthesis reaction by Superscript Reverse Transcriptase I (GIBCO BRL, MD) following

manufacturer's suggestion. Two microliters of cDNA were used in PCR reaction. The 5'-oligo was 5'-GAACACACTAGTACTATCCTGTGCCATTGCCATAGAGA-3' (SEQ ID NO: 33), and the 3'-oligo,

5 5'-GGAATATTGGGCCCTTGGATCCCAAGTCTGCACACCTGCACTCC-3' (SEQ ID NO: 34) with compatible restriction sites *SpeI* at 5'-end and *BamHI* at 3' end, respectively. After heat denaturation at 95°C for 2 minutes, 3-step cycling was performed at 45 seconds at 95°C, 45 seconds at 65°C and 60 seconds at 72°C by

10 Vent™ DNA polymerase (New England Biolabs, Beverly, MA). A final step for five minutes, at 72°C, was performed for end-polishing. The PCR product was digested by *SpeI* and *BamHI* (New England Biolabs) and ligated into the *SpeI*-*BamHI* sites of pBSKIIGH vector, containing the human growth hormone

15 fragment from pCIL-10 subcloned into the *BamHI*-*XhoI* site of pBSKII (Stratagene), which was named pBS-103L-GH. The pCIL-10 fragment containing human CD2 enhancer and Pμ promoter was then ligated immediately upstream of the 103L gene of pBS-103L-GH. MaxEfficient *E. coli* DH5α competent

20 cells (GIBCO BRL, MD) were used for transformation following manufacturer's suggestion. The transformants were grown in LB broth containing 0.1 μg/ml ampicillin and DNA were extracted by Qiagene Plasmid Maxi Kit (Qiagene, CA). Restriction analysis was performed for confirmation, and the

25 construct was sequenced to eliminate any possible PCR introduced mutations. A plasmid designated pCD2-103L-GH was selected for production of transgenic mice.

Production of Transgenic Mice

C3H/HEJ and FVB/NJ mice were obtained from the Jackson

30 Laboratory (Bar Harbor, ME). Females aged 3-4 weeks were induced to ovulate by intraperitoneal injection of pregnant mare's serum (PMS) between 10 a.m. to 2 p.m., followed 46 hours later by intraperitoneal injection of human chorionic gonadotropin (hCG). Following hCG administration, the

35 females were housed overnight with males of the same strain. The following morning females were examined for the presence of a copulation plug and embryos were isolated from those

females with plugs, essentially as described in *Manipulating the Mouse Embryo* (Hogan et al., eds., Cold Spring Harbor Laboratory Press, 1994).

DNA for embryo microinjection was prepared by digesting 5 of p200Tr3 and pCD2-103L-GH1 with NotI and XhoI followed by gel electrophoresis. The 9 kb and 10 kb fragments, respectively, were electrophoresed onto an NA-45 membrane (Schleicher and Schuell) by cutting a slit into the gel immediately in front of the desired band, inserting the NA-45 10 membrane and continuing electrophoresis until the DNA band has been transferred to the membrane. The DNA was eluted from the membrane by incubation with 0.4 ml of 1M NaCl/0.05M arginine-free base at 65-70°C for several hours in a microfuge tube. The eluted DNA was extracted with 15 phenol/chloroform and chloroform, ethanol precipitated and dissolved in 200 µl of 5 mM Tris, pH 7.5/0.1 mM EDTA. The DNA was then re-precipitated with ethanol and re-dissolved in 40 µl of 10 mM Tris, pH 7.5/0.1 mM EDTA. Prior to microinjection, the DNA was diluted to 1-2 µ/ml in 10 mM 20 Tris, pH 7.5/0.1 mM EDTA.

DNA was microinjected into the male pronuclei of strain C3H/HEJ or FVB/NJ embryos and injected embryos were transferred into the oviducts of pseudopregnant females essentially as described in *Manipulating the Mouse Embryo*. 25 The resulting offspring were analyzed for the presence of transgene sequences by Southern blot hybridization of DNA prepared from tail biopsies.

Southern blot analysis of transgenic mice:

Approximately 1/2" piece of tail was clipped and 30 digested in 500 µl proteinase K solution [containing 100 mM Tris HCl, pH 8.0; 5 mM EDTA, pH 8.0; 0.2% SDS; 200 mM NaCl; 100 µg/ml Proteinase K (Boehringer Mannheim, Germany)] at 55°C overnight. Digests were centrifuged for 15 minutes to remove undigested debris. Supernatants were precipitated 35 with an equal volume of isopropanol at room temperature. Precipitates were centrifuged for 25 minutes and pellets washed in 75% ethanol. Pellets were air dried and

resuspended in 100 μ l TE; pH 8.0. Restriction digests of tail DNA were performed as follows: 20 μ l DNA solution was digested with 80 units BamHI (New England Biolabs) in the presence of 1 mM spermidine overnight at 37°C. Digested samples were analyzed by gel electrophoresis using 0.8% agarose gels. Separated DNA was transferred to Hybond-N+ (Amersham, Inc.) following depurination in 0.25M HCl for 10 minutes followed by 0.5 M NaOH, 1 M NaCl for 30 minutes, and then 2.5M Tris-HCl (pH 7.4), 2.5M NaCl for 30 minutes. Immediately prior to transfer, gels were briefly equilibrated in a 10X SSC transfer buffer. Transfer was carried out overnight in 10X SSC by capillary action. After transfer, the membrane was air dried and UV-crosslinked using a Stratolinker (Stratagene, Inc.). After crosslinking, membranes were rinsed briefly in 2X SSC.

For 200 gene transgenic analysis, radiolabelled probe containing approximately 500 base pairs of the human IgM promoter was produced using the Random Primed DNA Labelling Kit (Boehringer Mannheim). The 500 bp Xba-1/Spe-1 fragment of human IgM heavy chain promoter was used as probe. Hybridization was carried out using standard hybridization procedures using Rapid-Hyb (Amersham) hybridization solution. 1 x 10⁶ cpm per ml of hybridization solution was incubated at 65°C overnight. Membranes were washed twice in 0.5X SSC 0.1% SDS at 65°C for 30 minutes and were exposed by autoradiography. Transgenic animals were detected by the presence of an approximately 7.0 kb BamHI fragment which hybridizes to a probe containing the 0.5 kb P μ fragment.

For 103 gene transgenic animals, a ³²P-radiolabelled PCR fragment of the pCD2-103L-GH construct described above was utilized. The PCR fragment was generated using the following primers: 5' oligo: 5'-GTA-AAT-CGT-CCT-GGG-GTC-TGG-3' (SEQ ID NO:35; 3' oligo: 5'-CCT-TCT-GAT-AAC-ACA-AGC-ATA-AAT-C-3' (SEQ ID NO:36). Using these oligonucleotide primers and the pCD2-103L-GH template, PCR reactions conditions were as follows: 20 cycles of 30 seconds at 94°C, 30 seconds at 60°C and 30 seconds at 72°, using Vent™ DNA polymerase (New

England Biolabs, Beverly MA). Upon hybridization to mouse genomic digested with EcoRI and SpeI, the resulting probe hybridized to an endogenous 2.4 kb band and a 0.85 kb transgenic-specific band.

5

11.2. RESULTS

200 gene transgenic mice (four C3H founder lines, 6 FVB founder lines) and 103 gene transgenic mice (five FVB founder lines) were produced according to the method
10 described above, in Section 11.1. Southern hybridization analysis demonstrated the successful production of both 200 and 103 gene transgenic founder animals.

With respect to the 200 transgenic animals, four lines of transgenic mice were created in the C3H inbred strain of
15 mice. One of these lines was examined for expression of the 200 transgene. As expected, 200 transgene transcripts were detected in the thymus, spleen and lymph nodes, consistent with a predominantly T-cell restricted pattern. At approximately 6 to 7 months of age, three of the founder
20 animals, upon visual examination, appeared sick. One of these founders, designated 130-1.2, was sacrificed at approximately 6 months of age. At the time the sacrifice, it was expected that the female would not have lived significantly longer. Upon dissection of 130-1.2, it was
25 clear that the spleen and one of the kidneys were grossly abnormal. The spleen was approximately ten-fold normal size and appeared to be filled with pale appearing cells. The splenocyte populations were examined by flow cytometry, and it was determined that the predominant cell population was
30 positive for MAC-1 (a macrophage/granulocyte cell surface marker) expression. These cells also had high side scatter profiles. Spleen sections from this animal were stained with hematoxylin and eosin and viewed by light microscopy. These data suggest that the abnormal cell population was composed
35 of polymorphonuclear neutrophils. The abnormal kidney also appeared to be infiltrated by these same cells.

One of the offspring of 130-1.2 died at approximately 6 months of age while giving birth to her second litter. Upon dissection, it was noted that there appeared to be a bowel obstruction, which may have contributed to the cause of death. In addition, yet another founder animal appeared to be quite sick and was sacrificed. However, in this animal there were no abnormalities observed, either by gross inspection of the organs or by flow cytometric analysis of lymphoid populations. Finally, the remaining founder animal was observed to be exhibiting symptoms of sickness by approximately 6 months of age.

Given that these animals were maintained under SPF (specific pathogen free) conditions, it is highly unlikely that these animals became ill via exposure to an infectious pathogen. Rather, it is most likely that the effect of the transgene is modulating some aspect of the immune system. Based on the observation of 130-1.2, it is suspected that as a consequence of transgene expression, the line may suffer from an immunodeficiency and is, therefore, susceptible to infection by normally innocuous organisms present in the environment (bacteria, etc.). It is possible, therefore, that this gene product normally functions in some aspect of the immune effector response or in the proper regulation of the immune system.

Two hundred transgenic mouse founder lines generated in the FvB inbred strain exhibited no outward symptoms of illness as they approached 6 months of age.

12. EXAMPLE: THE 103 GENE PRODUCT EXHIBITS A CRITICAL ROLE IN REGULATING TH2 EFFECTOR CELL RESPONSES

The Example herein presents in vivo data demonstrating that the 103 gene product regulates TH2 effector cell responses. In particular, a monoclonal antibody (3E10 mAb) has been generated against the 103 gene product, and its effect in an adoptive transfer model of TH1 and TH2 immune responses was investigated. The effect of a 103 gene product

fused to an Ig tail (103/Ig fusion) has also been studied in the adoptive transfer model.

The anti 103 gene product mAb abrogated the production of IL-4, IL-5, IL-6 and IL-13, TH2 mediated lung inflammation and the associated airway hyperresponsiveness. Likewise, the 103/Ig fusion results in a decrease in eosinophil infiltration into and inflammation of lung airways. In contrast, the 103 gene product mAb failed to inhibit TH1-mediated lung pathology and IFN- γ secretion.

These results, therefore, provide in vivo animal data indicating that the 103 gene product provides a critical signal to TH2 effector cells and can be utilized as a novel target for the selective suppression of TH2 immune responses.

12.1. MATERIALS AND METHODS

CD3/TCR Crosslinking: Mice expressing the transgene for the DO11.10 $\alpha\beta$ -TCR, which recognizes residues 323-339 of chicken ovalbumin (OVA) in association with I-A^d (Murphy, K.M., et al., 1990, Science 250:1720-1723) were utilized.

DO11.10 TCR-transgenic CD4⁺ T cells were cultured in complete RPMI 1640 with OVA₃₂₃₋₃₃₉ (1 μ M) and mitomycin C-treated splenocytes. For TH1 phenotype development, recombinant murine IL-12 (10ng/ml) and neutralizing anti-IL-4 mAb (11B11, 40 μ g/ml, R&D Systems) were added and for TH2 development recombinant murine IL-4 (10ng/ml) and neutralizing polyclonal anti-murine IL-12 (TOSH-2, 3 μ g/ml, Endogen, Cambridge, MA) were used. Cultures were maintained for 48 hours and 5 days after stimulation, after which time cells were harvested and purified over ficoll. 1 x 10⁷ cells were washed and RNA extracted as described below. The remainder of the cells were stimulated on plate bound anti-CD3 in the presence of h IL-2 (Endogen) for 48 hrs.

anti-103 Ab: Rat monoclonal antibodies (MAbs), including the 3E10 MAb, were generated against the extracellular domain of the mouse 103 gene product. A DNA sequence containing the extracellular domain of 103 gene

product was PCR-amplified and cloned into a vector containing the CD5 signal sequence and the human IgG1 constant region. COS cells were transiently transfected using lipofectaminetm (GIBCO) protocol according to manufacturer's instructions.

- 5 Cells were cultured in Ultra-Low[™] Ig fetal bovine serum (GIBCO) for approximately one week prior to harvest. and the recombinant protein was purified by passage over a protein A column.

Lou/M rats were then immunized by subcutaneous injection
10 of 0.5 mg purified recombinant 103 gene product protein. Rats were boosted twice via intraperitoneal injections at 2 week intervals with approximately 300 µg purified protein.

Animals were analyzed for reactivity to the fusion protein by FACS and ELISA approximately 10 days after the last boost.

- 15 Four weeks later, positive reacting animals were boosted once more and sacrificed 3 days later. Splenocytes were fused with SP/2 myeloma cells and resulting clones were screened and selected to be specific for the 103 gene product on the basis of their reactivity against 103 gene product Ig, but not
20 CD44-Ig, and their ability to stain 103 gene product COS transfectants, but not control transfectants. Pre-immune serum from non-immunized Lou/M rats was used as negative controls.

One of these mAbs was identified and termed 3E10.

25

Surface Expression Of 103 Gene Product On TH2 Clones And TH2 Effector Cells

- The 3E10mAb was labeled with digoxigenin and the number of 103-positive cells were detected by anti-digoxigenin Fab
30 fragments (Boehringer Mannheim) conjugated to Cy5.

- CD4 positive, L-selectin negative cells were isolated using high gradient magnetic cell separation system MACS (Milteny; Biotec, Berg-Gladbach). Expression of 103 was analyzed on a fluorescence-activated cell sorter (FACS)-
35 calibur (Becton-Dickinson) five to seven days after restimulation with OVA peptide under the indicated polarizing conditions.

In Vitro Activation Of TH2 Effector Cells

TH2 effector cells were activated with platebound CD3 (1 μ g/ml, 2C11) and CD28 (37.51, 4 μ g/ml), Pharmingen, San Diego) and 3E110 (20 μ g/ml) for 48 hours. IL-4 and IL-5 levels were measured in the supernatant by Elisa.

RNA isolation: Total cellular RNA for RT-PCR analysis was extracted from cells using the Rneasy Total RNA kit (Qiagen; Chatsworth, CA). Poly A+ RNA (for Northern analysis) was isolated from activated cells using FastTrack mRNA Kit (Invitrogen Corp.; San Diego, CA).

Northern Analysis: 1.0 μ g RNA were loaded per lane for the Northern blot analysis. The 103 gene probe was a 409 bp RsaI fragment from the 103 gene cDNA (position 1252-1661 based on the published sequence for Genbank accession number D13695). IL-4 and beta-actin probes were purchased from Clontech, Inc., Palo Alto, CA. IFN- γ probe consisted of a 344bp fragment of murine IFN- γ covering the region from position 532-876 (genbank accession number M28621). Northern blot analysis was carried out according to standard techniques.

RT-PCR: First strand cDNA was synthesized from equal amounts of RNA using the Superscript Preamplification System (Life Technologies; Gaithersburg MD). PCR was performed using 25 ng of first-strand cDNA. The following gene-specific primers were used for PCR amplification: Gene 103: 5' ACGGAGGGCAGTAAATC and 5' CAGCCAAGAAGTGAGAGC; IFN-gamma 5' TGTGCGCGAATCCAGCCTCAG and 5' GTCCCCCAGGAGATACAACC. Primers for glyceraldehyde 3- Phosphate Dehydrogenase (G3PDH) and IL-4 were purchased from Clontech Laboratories (Palo Alto, CA). PCR was carried out using the Advantage KlenTaq Polymerase mix (Clontech Laboratories; Palo Alto, CA) according to the provided protocol; annealing temperature 56 $^{\circ}$ C. Samples were removed from the PCR reaction beginning after 15 cycles and then after 5-cycle increments. Reactions

using the minimum number of cycles to visualize the gene of interest, were loaded onto 1.5% agarose gels for analysis.

TH recipient mice: TH1 and TH2 subsets were generated as described above. Mice were injected with 2×10^6 TH2 cells intravenously into recipient BALB/c mice. Twenty four hours later, mice were exposed daily to an aerosol of OVA (50 mg/ml) (Grade V, Sigma, St. Louis) for 20 min for 2 consecutive days. Control mice were either injected with TH2 cells and exposed to an aerosol of PBS or were exposed to OVA in the absence of cell transfer. Mice were sacrificed 24 hrs after the last aeroallergen challenge. One hr prior to allergen exposure, mice were injected with either 20 μ g or 100 μ g of 3E10 mAb, recombinant 103 gene product-IgG fusion protein, or 100 μ g of rat IgG1 (Sigma, St. Louis) as the appropriate isotype control. Twenty fours after the last challenge, the trachea was cannulated and a bronchoalveolar lavage performed with 4 x 0.3 ml aliquots of PBS (Gonazlo, J.A., et al., 1996, Immunity 4:1-14). Cytokine levels in the lavage fluid were measured by ELISA (PharMingen, San Diego).

Flow cytometry analysis of TH clones: AE7 (TH1), Dorris (TH1), DAX (TH2) and D10.G4 (TH2) clones were analyzed for the expression of gene 103 protein using fluorescence activated cell sorting (FACS). Cells were stimulated with appropriate antigen and cultured for approximately 3 days prior to analysis. Pre-immune serum was prepared for unimmunized Lou/M rats.

50 μ l of 3E10 culture supernatant (or 1 μ g purified 3E10 protein) was applied 1×10^6 cells. After rinsing, cells were contacted with goat anti-rat antibody conjugated with PE (R-phycoerythrin) fluorescent dye. After a final rinse, cell analysis was carried out on a FACS Vantage (Becton Dickenson).

35

103/Ig fusion protein: the 103/Ig fusion proteins were generated as discussed, above, in the Example presented in Section 10.

5 Animal Model Methods:

Cell Preparation and polarization: Spleens from DO11.11 OVA $\alpha\beta$ TCR mice were removed and CD4⁺ T cells were purified by negative selection. Cells were plated at a density of 1×10^6 /ml in 75 mm² flasks and stimulated with 10 μ g/ml OVA peptide and mitomycin C treated splenocytes at a ratio of 1:1 CD4: APC. Cells were cultured in the presence of IL-4 (20 ng/ml) and anti-IL-12 (3 μ g/ml) for TH2 polarization, or IL-12 (20 ng/ml) and anti-IL-4 (40 μ g/ml) for TH1 polarization. This procedure was repeated for 3 rounds of polarization.

15 Cells were then harvested, dead cells removed by density centrifugation. TH1 and TH2 cells were then incubated at 1×10^6 /ml for 48 hrs in IL-2 alone (10 ng/ml).

Adoptive transfer model: 2×10^6 cells were injected intravenously via the tail vein into recipient transgenic mice. Twenty four hours later, mice were exposed daily to an aerosol of OVA (50 mg/ml) antigen (Grade V, Sigma, St. Louis) for 20 minutes. Control mice were exposed to an aerosol of PBS alone. Mice were sacrificed on days 3, 5 and 7. In separate experiments, mice received 20 μ g/mouse i.v. of either 3E10 MAb or the 103 Ig fusion protein. Control mice were injected with 20 μ g of either rat or human Ig as the appropriate isotype control. This procedure was repeated for two consecutive days.

30 24 hours after the last challenge, mice were anaesthetized with 0.3 ml of 14% urethane i.p. and the trachea cannulated. A bronchoalveolar lavage (BAL) was performed by injecting 0.3 ml of PBS into the lungs. The fluid was then withdrawn and stored on ice. This procedure was repeated a total of 4 times. The cell suspension was then centrifuged (5 mins, 1500 rpm, 4° C) and the supernatant removed and frozen at -20° C. The cell pellet was then

resuspended in 1 ml of PBS and total cell counts were obtained. Cytospin preparations were then prepared and stained with Diff-Quik (Baxter Corporation). A total of 200 cells were then counted differentially using standard morphological criteria. Cytokine levels were measured in the BAL fluid by ELISA (Pharmingen, San Diego).

Active immunization protocol and IgE measurement: Male BALB/c mice (15-20 g) were immunized intraperitoneally with 7.5 μ g of OVA and 1.5 mg Al(OH)₃ in saline on Day 0 and Day 7. On day 14 and Day 21 the mice were challenged with aerosolized OVA (10 mg/ml) for 1 hours. Control mice were challenged with PBS instead of OVA. One hour prior to each allergen sensitization and challenge, the mice were injected with 100 μ g of 3E10 Mab or 100 μ g of rat IgG1 (Sigma, St. Louis). Twenty-four hours following the second allergen challenge a BAL was performed and IL-5 levels in the BAL fluid determined. Serum OVA specific IgE was determined by specific ELISA.

20

Airway responsiveness: Airway responsiveness was measured in TH2 recipient mice, 24 hours after the last aerosol challenge by recording respiratory pressure curves by whole body plethysmography (Hamelmann, J.E., 1997, Am. J. Respir. Crit. Care Med. 156:766-775); Buxco®, EMKA Technologies, Paris, France) in response to inhaled methacholine (Aldrich-Chemie, Steinheim, Germany) at a concentration of 2.5 to 25 mg/ml for 1 minute. This method allowed measurements of spontaneous breathing in a non-restrained mouse. Airway responsiveness was expressed in enhanced pause (Penh), a calculated value, which correlates with measurement of airway resistance, impedance and intrapleural pressure in the same mouse. $Penh = (Te/TR1) \times Pef/Pif$ (Te = expiration time, Tr = relaxation time, Pef = peak expiratory flow, Pif = peak inspiratory flow) (Hamelmann, J.E., 1997, Am. J. Respir. Crit. Care Med. 156:766-775).

Lung Histology: Following the BAL analysis, lungs were inflated with 0.6 ml of a mixture of OCT compound (Tissue-kek®; Miles Inc., Elkhart, IN) and 20% sucrose (Sigma, St. Louis, MI) at a ratio of 1:1. The lungs were then removed, 5 snap-frozen and 8-10 µm cryosections fixed in methanol at 20°C for 2.5 minutes. Slides were stained with haematoxylin and eosin (Fluka Chemika, Buchs, Switzerland).

In situ Hybridization: Recipient Balb/C mice were 10 injected intervenously with 2×10^6 TH1 or TH2 cells generated as described above. Twenty-four hours later, mice were exposed to an aerosol of OVA (50 mg/ml; Grade V, Sigma) for 20 minutes for two consecutive days. Mice were sacrificed 24 hours after the last aeroallergen challenge. Lungs were 15 removed and snap frozen for *in situ* hybridization. A 35-mer antisense oligonucleotide against 3'-UTR 103 gene sequence was synthesized and end-labeled as follows: 100 pmol oligo was incubated for 15 minutes at 37°C with 10 nmol dATP (Promega), 40 µmol biotin-dUTP, 1x terminal transferase 20 buffer, 5 mM CoCl_2 , 50 units transferase (Boehringer-Mannheim, Germany). Formalin fixed 5 µm tissue sections were hybridized for 16-18 hours. Control slides were hybridized with probe mix containing 50-fold excess unlabelled oligo. Hybridized probe was detected with a biotinyl tyramide 25 amplification method (GenPoint, Dako) and visualized by addition of AEC substrate kit (Vector) for five minutes.

12.2. RESULTS

RT-PCR analysis performed herein demonstrates that the 30 103 gene is induced only upon CD3/TCR crosslinking during differentiation of TH0 to TH2, but not TH1 effector cells. The RT-PCR analysis was confirmed by Northern analysis. These data corroborate the results presented in the Example of Section 7, above.

35 To further investigate the expression and role of the 103 gene product in TH cells, a monoclonal antibody (3E10

mAb) directed against the extracellular domain of the 103 gene product was prepared and characterized.

Flow cytometry data is presented in FIG. 25 which demonstrates that the 3E10 mAb recognizes and binds to 5 representative clones of the TH2 cell subpopulation (D10.G4; DAX), but not clones of the TH1 subtype (AE7; Dorris). For these experiments, cells were contacted with 3E10 MAb, preimmune serum (negative control) or a second antiserum (positive control; referred to as " α TH1 serum" for AE7 and 10 Dorris, and "rat α 103 serum" for D10.G4 and DAX). In contrast, this mAb failed to recognize resting or activated CD4+ (L-selectin-), CD8+, B cells or macrophage cells.

When TH1 cells (AE7, Dorris) were analyzed, the peaks for 3E10 MAb and the negative preimmune serum exhibited the 15 same very low level of staining as the negative control preimmune serum. No detectable 103 gene product is present, therefore, on the surface of the TH1 cells. In contrast, with TH2 cells (D10.G4, DAX), the 3E10 MAb peak shifted significantly to the right, demonstrating the presence of 103 20 gene product on the TH2 cell surface. It is noted that for each clone analyzed, the positive control peak is shifted well to the right of background levels, as expected.

In addition to the TH2-specific expression pattern observed in established TH clones as discussed above, 3E10 25 mAb staining and flow cytometry analysis was utilized to successfully demonstrate that 103 expression dramatically increases when freshly isolated TH cells are cultured under conditions that induce TH2 cell polarization, with the expression being dependent on the degree of differentiation 30 of the TH2 phenotype. Such an increase was not observed naive CD4+ (L-selection negative) under TH1 cell polarization conditions (i.e., TH1 effector cells derived from the TH precursor cells).

As shown in FIG. 26, pretreatment of TH2 recipient mice 35 with 3E10 mAb inhibited the secretion of IL-4, IL-5, IL-6 and IL-13 by greater than 90%. In particular, analysis of the cytokine profile in the BAL revealed high levels of IL-4, IL-

5, IL-6, IL-10 and IL-13 in TH2 recipient OVA challenged mice (closed bars). There was no detectable TH2 cytokines in the BAL fluid of mice that received TH2 cells and were not exposed to ovalbumin. Pretreatment with 3E10 mAb resulted in a dramatic reduction in IL-4, IL-5, IL-6 and IL-13, but had no effect on IL-10 levels in the BAL (open bars). OVA challenge of TH1 recipient mice resulted in high levels of IFN- γ in the BAL fluid (closed bars) that was not inhibited by 3E10 mAb (open bars).

10 These data show that the 103 gene is differentially expressed in a TH2-specific manner, thereby corroborating the results presented in the Example of Section 7, above. In addition, the data demonstrate the feasibility of using antibodies to separate TH2 subpopulation cells away from
15 other cell types, thereby modulating a TH cell subpopulation by changing the number of cells belonging to one TH cell subpopulation relative to that of another TH cell subpopulation.

An in vivo TH1 and TH2 adoptive transfer model (Cohn, L. et al., 1997, J. Exp. Med. 186:1737-1747) was used to address the role of the 103 gene product in TH cells. In this adoptive transfer animal model, aeroallergen provocation of TH1 or TH2 recipient mice results in TH effector cell migration to the airways and is associated with an intense
25 neutrophilic (TH1) and eosinophilic (TH2) lung mucosal inflammatory response. The model represents an accepted animal model for asthma, a TH2-like disorder. *In situ* hybridization revealed a marked upregulation of 103 gene mRNA positive cells in lungs from allergen or PBS provoked TH2
30 recipient mice. In marked contrast there was no detectable 103 gene mRNA expression in lungs obtained from either OVA or PBS provoked TH1 recipient mice.

The animal model was used to investigate whether neutralization of the 103 gene product in vivo also abrogated
35 TH2-mediated pathology. Allergen provocation of mice which had received TH2 cells and control rat Ig resulted in infiltration of lymphocytes and eosinophilic inflammation of

the airways. In vivo administration of 3E10 mAb markedly suppressed the development of eosinophilic inflammation of the airways. In particular, eosinophilic inflammation was assessed, first, by histological analysis of the airway
5 tissue. Second, an analysis of the cellular composition of the bronchoalveolar lavage fluid (BAL) was performed (FIGS. 27A-27B). No significant reduction in the number of antigen specific TH2 cells that migrated into the airway interstitium after allergen challenge was observed.

10 In marked contrast to the effects on TH2 immune responses, 3E10 treatment did not suppress IFN- γ secretion or neutrophilic lung inflammation induced by allergen challenge of TH1 recipient mice. It is also of interest to note that the anti-103 gene product mAb failed to inhibit IL-10
15 secretion, a cytokine that has been shown to suppress eosinophil infiltration and prevent IgE mediated mast cell activation.

TH2 mediated lung mucosal eosinophilic inflammation is associated with heightened airway responsiveness to non
20 specific stimuli and is a characteristic feature of bronchial asthma (Ohashi, Y. et al., 1992, Am. Resp. Dis. 145:1469-76). To determine whether the 103 gene product is involved in this physiological consequence of allergen exposure, the degree of airway constriction induced by the methacholine inhalation
25 was assessed using whole body plethysmography.

3E10 mAb treatment was, indeed, demonstrated to attenuate allergen induced heightened airway responsiveness. In particular, 3E10 mAb treatment suppressed the development of airway hyperresponsiveness induced by OVA challenge in TH2
30 recipient mice (FIG. 28). TH effects of treatment with 3E10 mAb were comparable to those previously reported using anti-IL-5 mAbs Wang, L.M., 1992, EMBO 11:4899-4908 and anti-B7-2 mAbs (Tsuyuki, S. et al., 1997, J. Exp. Med. 185:1671-1679).

The role played by 103 gene in lung inflammation was
35 also investigated in an active immunization model where mice were injected systemically with antigen and adjuvant prior to two repeated allergen provocations. As summarized in FIGS.

29A-29B, administration of either 3E10 mAb or 103/Ig fusion results in a significant reduction in eosinophilic inflammation, as well as a significant reduction in IL-4 and IL-5 cytokine levels in the lung, which represent cytokine hallmarks of activated TH2 cell subpopulations. In addition, 3E10 mAb attenuated the induction of OVA specific IgE in the serum of the active immunization model. Still further, an approximate 60% reduction in airway hyperresponsiveness was observed after repeated aerosol challenge after active immunization.

Further, the level of interferon gamma was measured, which represents a hallmark of TH1 cell subpopulation activation, and an increase in its level was detected. This indicates the presence of a relative increase in TH1 cell subpopulation responses.

In addition, animals treated with a soluble fusion protein containing the extracellular domain of the 103 gene product fused to an Ig tail (103/Ig fusion). Administration of the 103/Ig fusion results in significant decrease in hallmark symptoms of asthma. As summarized in FIG. 29B, such administration results in animals that exhibit a decrease in eosinophil infiltration into lung airways (this was assessed by both BAL and histological examination). Likewise, administration of the 103/Ig fusion resulted in a 50% attenuation in the degree of eosinophilic inflammation of airways.

Thus, the inhibition of 103 gene function appears to modulate TH cell subpopulations by decreasing the level and/or activity of TH2 cells while bringing about a relative increase in the level and/or activity of TH1 cells.

To determine whether signalling through the 103 gene product directly modifies cytokine production, TH2 effector cells were activated with plate bound CD3 and CD28. Under conditions where Fc crosslinking occurred, 3E10 mAb augmented IL-4 and IL-5 secretion in the absence of enhanced proliferation (FIG. 30). In contrast, CD3/CD28 stimulation of TH1 cells in the presence of plate bound 3E10MAb failed to

modify IFN- γ secretion. These results indicate that ligation of the 103 gene product in conjunction with signals delivered through the CD3/CD28 complex together with CD28 mediated co-stimulation provide a novel costimulatory signal specific for 5 TH2 effector cells.

Recently, GATA-3 have been shown to be preferentially expressed in TH2 cells and suggested to play an important role in TH2 differentiation (Zheng, W.-P. & Flavell, R.A., 1997, Cell 89:587-596). Unlike GATA-3, however, the 103 gene 10 product is induced upon CD3/TCR mediated activation and not during TH2 differentiation from TH0 cells. GATA-3 may be involved in TH differentiation, while the 103 gene product may be more involved during activation of TH2 effector cells. Further, the 103 gene promoter in murine mast cells contains 15 a GATA-3 consensus binding sequence (Gachter, T. et al., 1996, J. Biol. Chem. 271:124-129), indicating that GATA-3 may be involved in the TH2 specific expression of the 103 gene.

In summary, these results provide both in vitro characterization of 103 gene expression and the 103 gene 20 product, as well as in vivo animal data indicating that the 103 gene product provides a critical signal to TH2 effector cells and represents a critical regulatory molecule for both cellular and humoral allergic inflammation. These data indicate that the 103 gene and/or gene product can be 25 utilized as a novel target for the selective suppression of TH2 immune responses.

30 13. EXAMPLE: THE 200 GENE PRODUCT EXHIBITS A CRITICAL ROLE IN THE RESOLUTION OF INJURY FOLLOWING KIDNEY ISCHEMIA AND REPERFUSION

The Example provided herein presents in vivo data that the 200 gene product is involved in the recovery from kidney ischemia injury. In particular, a monoclonal antibody (96.3.8H7 mAb) generated against the extracellular domain of 35 the murine 200 gene product, and its effect in a surgical model of recovery from kidney ischemia injury was

investigated. The anti-200 mAb markedly impaired restoration of renal function, as determined by several indicators.

These results, therefore, provide *in vivo* animal data demonstrating that the 200 gene product provides a critical function in the resolution of injury following kidney ischemia. Thus, the 200 gene and its gene product can be used to aid in the recovery from ischemic injuries such as stroke, heart attack, acute renal failure, and organ transplant.

10

13.1. MATERIALS AND METHODS

anti-200 mAb: Rat monoclonal antibodies (mAbs) were generated against the extracellular domain of the mouse 200 gene product. Recombinant murine 200 gene IgG1 (m200Ig) fusion protein was produced and purified as described in Section 10.1 above. Briefly, a DNA sequence containing the extracellular domain of the mouse 200 gene product was PCR-amplified and cloned into a vector containing the CD5 signal sequence and the human IgG1 constant region. COS cells were transiently transfected using lipofectamine™ (GIBCO) according to the manufacturer's instructions. Cells were cultured in Ultra-Low™ Ig fetal bovine serum (GIBCO) for approximately one week prior to harvest, and the recombinant protein was purified by passage over a protein A column.

Lu/M rats (Harlan Sprague Dawby, Inc., Indianapolis, IN) were immunized by subcutaneous injection of 600 mg of purified recombinant m200Ig fusion protein with 600 µl of complete Freund's adjuvant. Rats were boosted twice via intraperitoneal injections at two week intervals. The first boost consisted of 500 mg of m200Ig with incomplete Freund's adjuvant, and the second boost consisted of 500 mg m200Ig in the absence of Freund's. Animals were analyzed for reactivity to the fusion protein by FACS and ELISA 10 days after the last boost. Four weeks later, a selected animal which demonstrated positive reactivity to the m200Ig fusion protein was boosted once more with 500 mg of m200Ig and sacrificed three days later. Splenocytes from this animal

were fused with SP2/0 myeloma cells, and resulting clones were screened and selected to be specific for the murine 200 gene product on the basis of their reactivity to clonal TH2 cell lines (DAX, and D10.G4) and thymocytes from m200

5 transgenic mice versus non-transgenic mice. A single hybridoma, termed 96.3.8H7, was identified which selectively reacted to all TH1 cell lines and m200 transgenic thymocytes, but did not react to TH2 cell lines and non-transgenic thymocytes.

10

Surgical Methods: Mice were anesthetized with a single cocktail of Ketamine (200 mg/kg) plus Xylazine (10 mg/kg) injected intraperitoneally. 1 ml of 0.9% dextrose saline was also administered subcutaneously. Core body temperature was
15 maintained at around 37 °C. Using a midline abdominal incision renal arteries and veins were bilaterally occluded for 32 minutes with microaneurysm clamps, during which time the abdomen was closed. After the renal pedicle clamps were removed, the kidneys were observed for an additional five
20 minutes to document color change indicating reflow, and the incision was sutured. For controls, sham surgery was also performed on animals, wherein the surgical procedure was identical except that the microaneurysm clamps were not applied.

25 For 200 gene product blockage experiments, mice were pretreated 24 hours before surgery with 100 µg/mouse of rat anti-200 monoclonal antibody (200 mAb). In control experiments, mice were administered equivalent dosages of rat Ig (RtIg) antibody.

30 Mice were then given antibody at 24 hour intervals following surgical recovery (4 hours post anesthetic).

Animals were sacrificed at various periods following reperfusion, specifically at 12, 24, 48, 72, and 96 hours. For the later groups (24, 48, 72, and 96 hours post
35 reperfusion) mice were housed in metabolic cages for 24 hours prior to sacrifice in order to collect urine for protein analysis as a measure of renal dysfunction. At sacrifice,

the animals were exsanguinated by cardiac puncture under terminal anesthesia (induced by carbon dioxide in a precharged chamber) and the kidneys were removed. Serum was analysed for urea nitrogen and creatinine by Tufts Veterinary
5 Diagnostic Laboratories. One half of each kidney was frozen in liquid nitrogen for RNA extraction, one quarter was fixed in 10% buffered formalin for histological assessment by Hematoxylin and eosin, and the remaining quarter was frozen for in situ hybridization and immunohistochemical analysis.

10

Kidney Histology: Kidneys were processed, sectioned and stained with hematoxylin and eosin according to standard methods. The resulting sections were examined blind and were assigned an arbitrary score based upon the presence or
15 absence of the following histological parameters: focal interstitial inflammatory infiltrate, diffuse interstitial inflammatory infiltrate, glomerular leukocytic infiltrate, protein deposits in the tubule, presence of apoptotic bodies in the tubular epithelium, flattened or denuded tubular
20 epithelium, cellular debris in tubules.

13.2. RESULTS

The 200 gene product is similar to the rat adhesion molecule called KIM-1 (Ichimura et al., 1998, *J. Biol. Chem.*
25 273:4135), levels of which are increased in cells after ischemic/reperfusion injury. The role of the 200 gene product in ischemic injury was investigated in vivo using a mouse model of acute renal failure. In particular, this animal model was used to investigate whether neutralization
30 of the 200 gene product in vivo affected tissue repair after an ischemic injury.

A monoclonal antibody (96.3.8H7 mAb) directed against the extracellular domain of the 200 gene product was prepared and characterized, and this antibody was administered to mice
35 24 hours before, and at 24 hour intervals following ischemic kidney injury. Control animals were administered equal dosages of rat Ig at the same intervals. A second group of

animals underwent sham surgery, described in Section 13.1 above, wherein the animals were not subjected to ischemic kidney injury.

Serum creatinine and blood urea nitrogen (BUN) levels were measured in animals sacrificed 24, 48, 72, and 96 hours after kidney reperfusion. These levels are shown in Table 3 below. Creatinine and blood urea nitrogen levels returned to basal levels in untreated (+RtIg) mice within 72 hours post ischemia. However, mice treated with anti-200 mAb (+a200) maintained elevated levels of both blood urea nitrogen (122.5 mg/dl vs. 34.3 mg/dl) and creatinine (0.73 mg/dl vs. 0.3 mg/dl).

15

20

25

30

35

Table 3

	Group	n	Time Point	BUN (mg/dl)	Serum Creatinine (mg/dl)
5	I + RtIg	4	24-hr	39.75 ± 4.77	0.33 ± 0.06
	I + AB	9	24-hr	105.33 ± 15.53	1.02 ± 0.21
	S + RtIg	4	24-hr	20.25 ± 2.93	0.28 ± 0.02
	S + Ab	4	24-hr	29.50 ± 6.08	0.28 ± 0.02
10	I + RtIg	4	48-hr	135.00 ± 61.01	1.13 ± 0.49
	I + AB	11	48-hr	163.82 ± 36.30	1.76 ± 0.48
	S + RtIg	4	48-hr	26.25 ± 3.73	0.30 ± 0.00
	S + Ab	4	48-hr	28.50 ± 3.20	0.28 ± 0.03
15	I + RtIg	5	72-hr	37.60 ± 2.23	0.34 ± 0.02
	I + AB	8	72-hr	151.88 ± 54.45	1.09 ± 0.33
	S + RtIg	3	72-hr	35.00 ± 5.51	0.27 ± 0.03
	S + Ab	4	72-hr	28.50 ± 1.04	0.30 ± 0.00
20	I + RtIg	2	96-hr	51.50 ± 23.50	0.35 ± 0.05
	I + AB	5	96-hr	71.80 ± 16.09	0.46 ± 0.08
	S + Ab	1	96-hr	33.00	0.3

FIG. 31 shows histological cross sections of kidney tissue from untreated (Fig. 31A) and treated (Fig. 31B) rats at 72 hours post reperfusion. Treated mice still exhibited severe tissue damage with flattened tubular epithelial cells, leukocyte infiltration, and tubular cast. The mean histological score was +6 in anti-200 treated mice compared to control mice at 72 hours (Fig. 32).

In summary, these results provide *in vivo* animal data demonstrating that the 200 gene product plays a critical role during recovery from ischemic kidney injury, and can be used as a novel treatment to aid in the recovery from ischemic injuries such as acute renal failure.

14. EXAMPLE: THE 103 GENE PRODUCT IS EXPRESSED
IN HUMAN MAST CELLS

The example provided herein presents data demonstrating that the 103 gene product is expressed, in mammalian mast cells. First, Northern analysis showed high levels of 103 gene expression in a human mast cell line. The example also describes the production of monoclonal antibodies which are specific for the human, but not mouse, 103 gene product. FAC staining of the human mast cell line demonstrated binding of these monoclonal antibodies, confirming that the 103 gene product is, indeed, expressed in mast cells.

14.1. MATERIALS AND METHODS

103 fusion proteins: Monoclonal antibodies (mAbs; see below) were generated against the extracellular domain (amino acid residues 18-323) of a human 103 gene product (with a glutamic acid at amino acid residue 78). In particular, the following fusion protein was utilized: a fusion protein that contained, from amino- to carboxy-terminus, a CD5 signal sequence (CD5ss) plus GT residues, the extracellular domain of the 103 gene product described above.

A second Fc fusion protein of the human 103 gene product was constructed, according to the techniques described in Section 10.2 above, to bind ELISA plates for screening. In particular, the protein contain, from amino- to carboxy-terminus, a T075 signal sequence (T075ss) plus QR residues, amino acid residues 20-323 of the human 103 gene product plus a linker amino acid sequence (Ala-Ala-Ala-Asp-Pro) and a human IgG1 constant region.

A fusion protein of the mouse 103 gene product was also utilized that contained, from amino- to carboxy-terminus, a CD5 signal sequence plus GT residues, residues 24 (Thr) to 328 (Pro) of the mouse 103 gene product, and human IgG1 constant region.

A control fusion protein were also utilized which contained unrelated proteins (T001, a chemokine, or T075, a

tumor necrosis family receptor) fused with a human IgG1 constant region.

The human IgG1 sequence was as in Aruffo et al., 1990, Cell 61:1303.

5

Anti-103 mAb Generation: MAbs were generated in Balb/c mice against the extracellular domain (amino acid residues 18-323) of a human 103 gene product (with a glutamic acid at amino acid residue 78) utilizing the above-described fusion protein, coupled with standard techniques (see Section 5.6, above) for mAb generation, selection and purification.

Splenocytes from animals which showed positive reactivity to the 103 fusion protein were fused with SP2/0 myeloma cells, and the resulting clones were screened and selected to be specific for the human 103 gene product by ELISA and by Biacore (BIAcore, Inc.; Uppsala, Sweden) as described in Fagerstam et al., 1992, *J. of Chromatography* 597:397-410 and in Kretschmann & Raether, 1968, *Z. Naturforschung Teil. A.* 23:2135. Twenty-one clones were shown to specifically bind the human 103 gene product (with either alanine or glutamic acid at amino acid residue 78) but not to a mouse 103-gene product Fc fusion protein or to a control Fc fusion protein.

Northern Blots: Northern procedures performed in the experiments described in this example were performed as described, above, in Section 6.1.

Flow cytometry analysis of mast cell lines: Cells were analyzed for the expression of gene 103 protein using fluorescence activated cells sorting (FACS) according to standard methods described in Section 12.1, above using anti-mouse IgFITC secondary antibodies.

35

14.2. RESULTS

Northern blot analysis of multiple cells lines showed high levels of the 103 gene in a human mast cell line.

Expression of the 103 gene product in this cell line was verified using monoclonal antibodies raised against an Fc fusion protein of the human 103 gene product, as described in Section 14.1, above.

FACS staining of the human mast cell line with the 21 monoclonal antibodies showed staining with 15 of the 21 antibodies compared to isotype controls. Five of these 15 antibodies, identified as 1B4, 2O3, 3F7, 3H18, and 10F7, were selected for further analysis. FAC staining with these antibodies was demonstrated to be specifically blocked with an excess of human 103-Fc fusion protein, however, staining was not blocked with control Fc fusion proteins.

In summary, these results provide evidence that the 103 gene product is expressed in a human mast cell line. Accordingly, the 103 gene, its gene product, and compositions derived therefrom (*e.g.*, antibodies and other compounds which bind to and/or modulate the expression or activity of the 103 gene or its gene product) may be used not only in the treatment and regulation of TH2 immune disorders such as asthma, but may also be used in the treatment and regulation of mast cell related disorders. Such mast cell related disorders include, but are not limited to, atherosclerosis (see, *e.g.*, Metzler and Xu, 1997, *Int. Arch. Allergy Immunol.* 114:10-14) and myocardial ischemia/reperfusion (see, *e.g.*, Frangogiannis et al., 1998, *Circulation* 98:699-710).

30

15. DEPOSIT OF MICROORGANISMS

The following microorganisms were deposited with the Agricultural Research Service Culture Collection (NRRL), Peoria, Illinois, on January 19, 1995 (10-C, 57-E, 105-A, 106-H, 161-G, 200-O), March 3, 1995 (*E. coli* DH10B(Zip)TM containing 200-P) and June 6, 1995 (200-AF, 10-X, 54-C) and assigned the indicated accession numbers:

	<u>Microorganism</u>	<u>NRRL Accession No.</u>
	10-C	B-21390
	57-E	B-21391
	105-A	B-21392
5	106-H	B-21393
	161-G	B-21394
	200-O	B-21395
	<u>E. coli</u>	B-21415
	DH10B(Zip) TM	
10	containing 200-P cDNA	
	200-AF	B-21457
	10-X	B-21455
	54-C	B-21456

- 15 The following microorganisms were deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Virginia, on December 12, 1995 and assigned the indicated accession numbers:

20	<u>Microorganism</u>	<u>ATCC Accession No.</u>
	<u>E. coli</u> , feht 200C	69967

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended
 25 as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in
 30 the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

MICROORGANISMS	
Optional Sheet in connection with the microorganism referred to on pages __, lines __ of the description *	
A. IDENTIFICATION OF DEPOSIT * Further deposits are identified on an additional sheet *	
Name of depositary institution * American Type Culture Collection	
Address of depositary institution (including postal code and country) * 10801 University Blvd. Manassas, VA 20110-2209 US	
Date of deposit * Decemeber 12, 1995 Accession Number * 69967	
B. ADDITIONAL INDICATIONS * (leave blank if not applicable). This information is continued on a separate attached sheet	
C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE * (if the indications are not all designated States)	
D. SEPARATE FURNISHING OF INDICATIONS * (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later * (Specify the general nature of the indications e.g., "Accession Number of Deposit")	
E. <input checked="" type="checkbox"/> This sheet was received with the International application when filed (to be checked by the receiving Office) <div style="text-align: right;">Jeryl McDowell PCT/International Appl Processing Div. (703) 335-7839 (Authorized Officer)</div> <input type="checkbox"/> The date of receipt (from the applicant) by the International Bureau * was _____ <div style="text-align: right;">_____ (Authorized Officer)</div>	

Form PCT/RO/134 (January 1981)

Form PCT/RO/134 (cont.)

**Agricultural Research Culture Collection (NRRL)
International Depository Authority**

1815 N. University Street
Peoria, IL 61604
US

<u>Accession No.</u>	<u>Date of Deposit</u>
B-21390	January 19, 1995
B-21391	January 19, 1995
B-21392	January 19, 1995
B-21393	January 19, 1995
B-21394	January 19, 1995
B-21395	January 19, 1995
B-21415	March 3, 1995
B-21455	June 6, 1995
B-21456	June 6, 1995
B-21457	June 6, 1995

WHAT IS CLAIMED IS:

1. A method for ameliorating a symptom of an ischemic disorder or injury in a mammal, comprising administering to
5 the mammal a 200 gene product in an amount effective to ameliorate the symptom of the ischemic disorder or injury.
2. A method for ameliorating a symptom of an ischemic disorder or injury in a mammal, comprising administering to
10 the mammal a nucleic acid molecule encoding a 200 gene product in an amount effective to ameliorate the symptom of the ischemic disorder or injury.
3. A method for ameliorating a symptom of an ischemic
15 disorder or injury in a mammal, comprising administering to the mammal an antibody directed against a 200 gene product in an amount effective to ameliorate the symptom of the disorder.
- 20 4. The method of Claim 1, 2, or 3, wherein the ischemic disorder is ischemic renal disease, or myocardial ischemia.
5. The method of Claim 4, wherein the myocardial
25 ischemia is angina pectoris.
6. The method of Claim 1, 2, or 3 wherein the ischemic disorder or injury is a infarction.
- 30 7. The method of Claim 6, wherein the infarction is a myocardial infarction, or a cortical infarction.
8. The method of Claim 1, 2, or 3, wherein the
ischemic injury is to a transplanted organ.
- 35 9. The method of Claim 8, wherein the transplanted organ is a kidney.

10. The method of Claim 1, 2, or 3, wherein the 200 gene product is a polypeptide comprising:

- (a) the amino acid sequence of SEQ ID NO:10,
- (b) the amino acid sequence encoded by the nucleotide
5 sequence of SEQ ID NO:8,
- (c) the amino acid sequence encoded by the cDNA insert
of the clone *E. coli* DH10B(Zip)[™] containing 200-P
(NRRL Accession No. B-21415), 200-AF (NRRL
Accession No. B-21457), or 200-O (NRRL Accession
10 No. B-21395),
- (d) the amino acid sequence of SEQ ID NO:24,
- (e) the amino acid sequence encoded by the nucleotide
sequence of SEQ ID NO:37, or
- (f) the amino acid sequence encoded by the cDNA insert
15 of the clone feht200C (ATCC Accession No. 69967).

11. The method of Claim 1, 2, or 3, wherein the 200 gene product is a polypeptide encoded by a nucleic acid molecule which hybridizes under highly stringent conditions
20 to the complement of:

- (a) a nucleic acid molecule which encodes the amino
acid sequence of SEQ ID NO:10;
- (b) a nucleic acid molecule comprising the nucleotide
sequence of SEQ ID NO:8,
- 25 (c) the cDNA sequence contained in the clone *E. coli*
DH10B(Zip)[™] containing 200-P (NRRL Accession No. B-
21415), 200-AF (NRRL Accession No. B-21457), or
200-O (NRRL Accession No. B-21395),
- (d) to the complement of a nucleic acid molecule which
30 encodes the amino acid sequence of SEQ ID NO:24,
- (e) to the complement of the nucleotide sequence of SEQ
ID NO:37, or
- (f) to the complement of the cDNA sequence contained in
the clone feht200C (ATCC Accession No. 69967).

35

12. The method of Claim 2 wherein the nucleic acid molecule encoding a gene 200 product comprises:

- (a) a nucleotide sequence which encodes the amino acid sequence of SEQ ID NO:10,
- 5 (b) the nucleotide sequence of SEQ ID NO:8,
- (c) the nucleotide sequence of the cDNA insert of the clone *E. coli* DH10B(Zip)[™] containing 200-P (NRRL Accession No. B-21415), 200-AF (NRRL Accession No. B-21457), or 200-O (NRRL Accession No. B-21395),
- 10 (d) a nucleotide sequence which encodes the amino acid sequence of SEQ ID NO:24,
- (e) the nucleotide sequence of SEQ ID NO:37, or
- (f) the nucleotide sequence of the cDNA insert of the clone feht200c (ATCC Accession No. 69967).

15

13. The method of Claim 1 wherein said administering of the 200 gene product is parenteral, subcutaneous, intraperitoneal, intrapulmonary, intranasal, or intralesional.

20

14. The method of Claim 13, wherein the intralesional administration comprises perfusing or contacting a graft or organ with the 200 gene product before transplant.

25

15. The method of Claim 2 wherein said administering of the nucleic acid is parenteral, subcutaneous, intraperitoneal, intrapulmonary, intranasal, or intralesional.

30

16. The method of Claim 15, wherein the intralesional administration comprises perfusing or contacting a graft or organ with the nucleic acid before transplant.

17. The method of Claim 3 wherein said administering of
35 the antibody is parenteral, subcutaneous, intraperitoneal, intrapulmonary, intranasal, or intralesional.

18. The method of Claim 17, wherein the intralesional administration comprises perfusing or contacting a graft or organ with the antibody before transplant.

5 19. The method of Claim 3, wherein the amount of the antibody administered is from about 1 μ g/kg to about 100 mg/kg.

20. The method of Claim 19, wherein the amount of the
10 antibody administered is from about 1 μ g/kg to about 15 mg/kg.

21. The method of Claim 20, wherein the amount of the
antibody administered is from about 0.1 mg/kg to about 2.0
15 mg/kg.

20

25

30

35

1 / 47

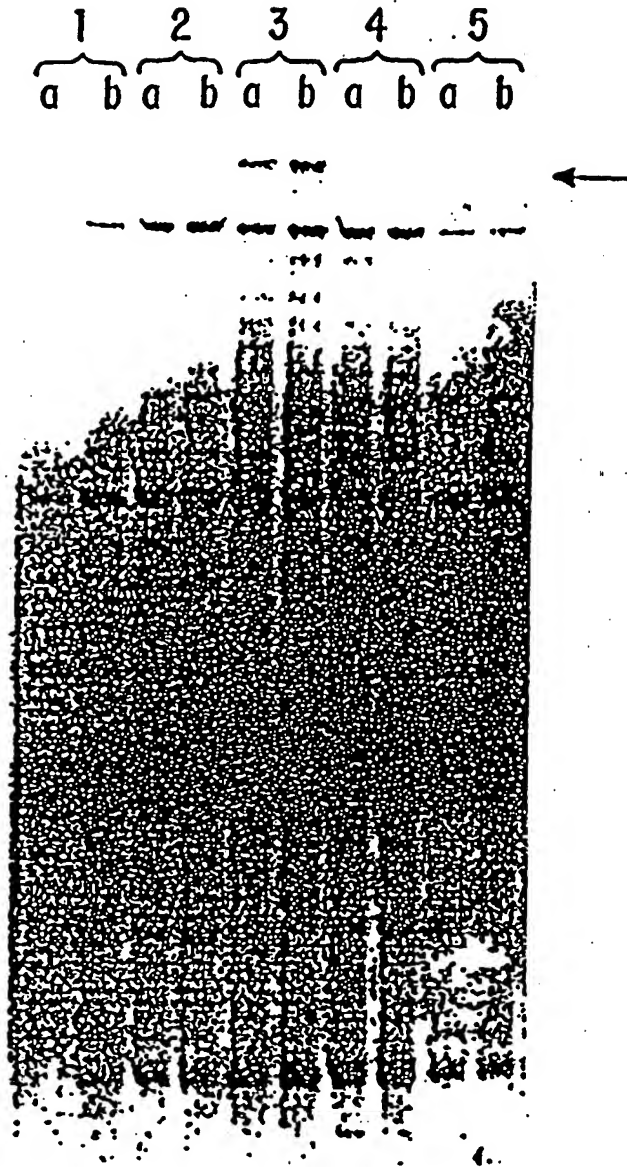


FIG. 1

2 / 47

10	20	30	40	50	60	
CTGGTGAGGG	GGATCTACAA	CTTGTTCCGGT	TAAAGAAAAA	AGCAACAGCC	AACAGAAATG	60
TGGTTATCCT	TCACCTACCT	AAAAAGGGAG	ATGATGTGAA	ACCAGGAACC	AGATGCCGAG	120
TAGCAGGATG	GGGGAGATTT	GGCAATAAGT	CAGCTCCCTC	TGAAACTCTG	AGAGAAGTCA	180
ACATCACTGT	CATAGACAGA	AAATCTGCA	ATGATGAAAA	ACACTATAAT	TTTCATCCTG	240
TAATTGGTCT	AAACATGATT	TGGGCAGGGG	ACCTCCCCGG	CGGAAAGGAC	TCCTGCAATG	300
GGGATTCTGG	CAGCCCTCTC	CTATGTGATT	GGTATTTGGG	AGCATCACC	TCCTTTT	357

FIG. 2

10	20	30	40	50	60	
TTAGCGCCAT	TGCCATAGAG	AGACCTCAGC	CATCAATCAC	TAGCACATGA	TTGACAGACA	60
GAG/MTGGGA	CTTTGGGCTT	TGGC/MTTCT	GACACTTCCC	ATGTATTTGA	CAGTTACGGA	120
GGGCAGTAAA	TGTCCTGGG	GTCTGG/AAA	TGAGGCTTTA	ATTGTGAGAT	GCCCCAAAG	180
AGGACGCTCG	ACTTATCCTG	TGGAATGGTA	TFACTCAGAT	ACAAATGAAA	GTATTCCTAC	240
CCAAAA/AAAA	AAAAA					255

FIG. 4A

3 / 47

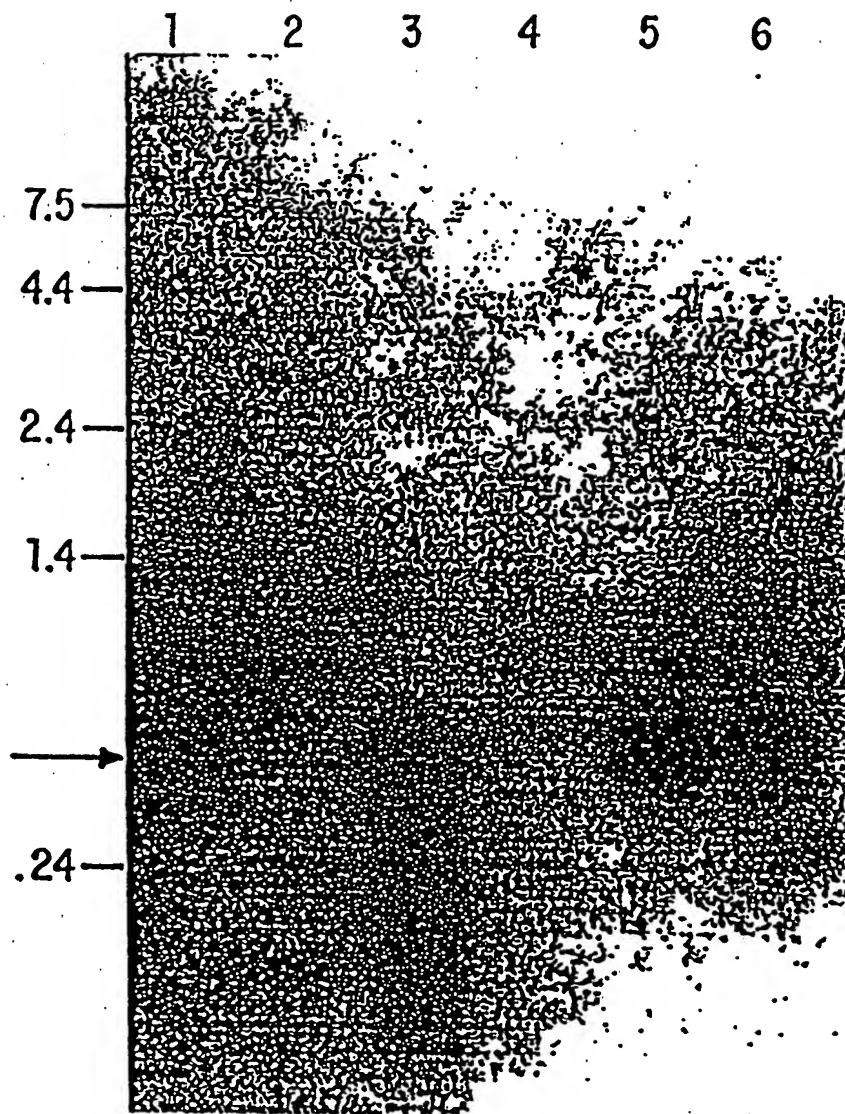


FIG. 3

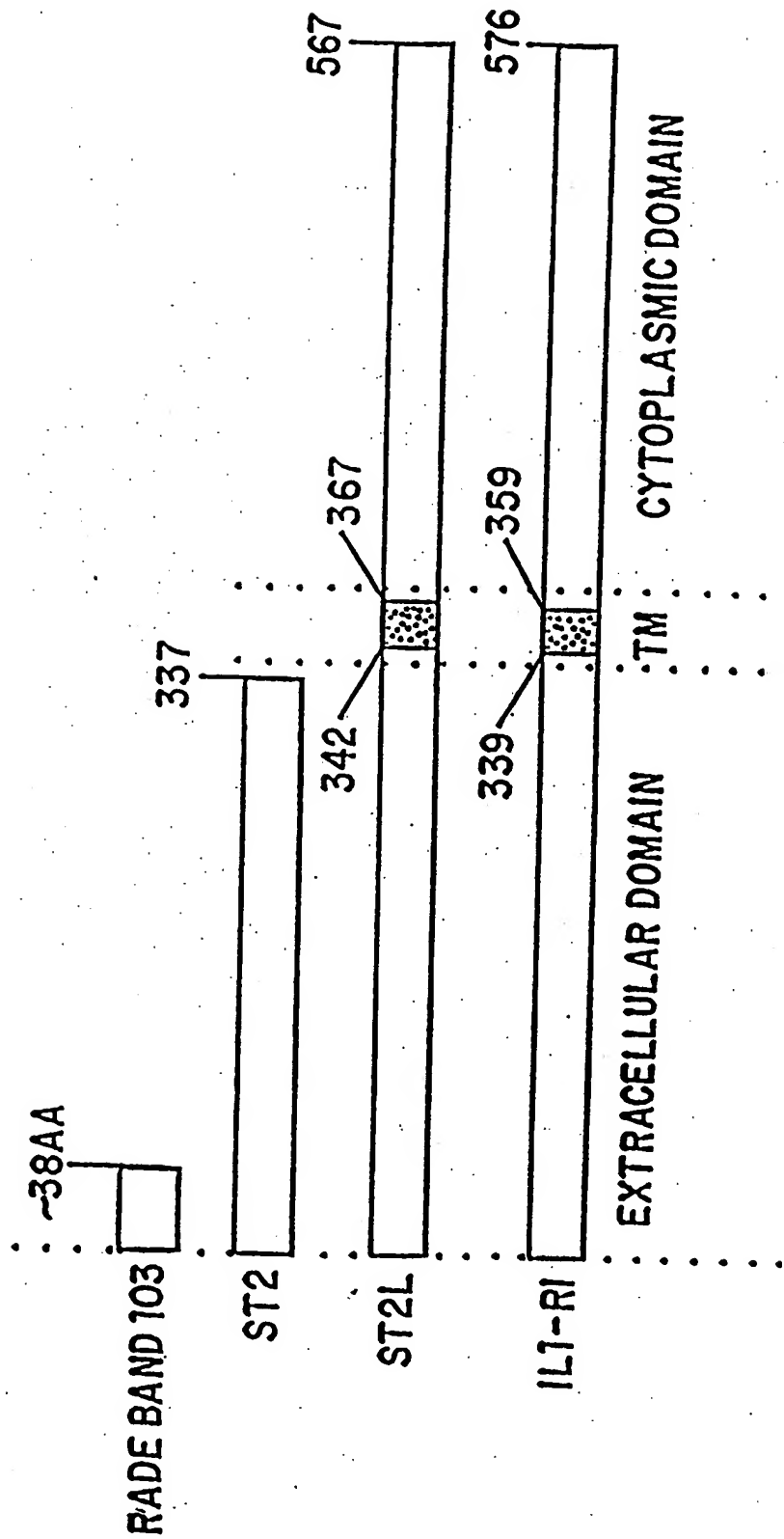


FIG. 4B

5 / 47

1 atgattgaca gacagagaat gggactttgg gctttggcaa tictgacact tcccatgtat
61 ttgacagtta cggagggcag taaatcgctc tggggctcgg aaaatgaggc ttaattgtg
121 agatgcccc aaagaggacg ctcgacttat cctgtggaat ggtattactc agatacaaat
181 gaaagtattc ctactcaaaa aagaaatcgg atctttgtct caagagatcg tctgaagtti
241 ciaccagcca gagtgaaga ctctgggatt tatgcttggt ttatcagaag ccccaacttg
301 aataagactg gatacttgaa tgtcaccata cataaaaagc cgccaagctg caatatccct
361 gattatttga tgtactcgac agtacgtgga tcagataaaa attcaagat aagctgtcca
421 acaattgacc tgtataattg gacagcacct gttcagtggg ttaagaactg caaagctctc
481 caagagccaa ggttcagggc acacaggctc tacttgttca ttgacaacgt gactcatgat
541 gatgaagggt actacacttg tcaattcaca cacgcggaga atggaaccaa ctacatcggt
601 acggccacca gatcattcac agttgaagaa aaaggctttt ctatgtttcc agtaattaca
661 aatcctccat acaaccacac aatggaagtg gaaataggaa aaccagcaag tattgcctgt
721 tcagcttgct ttggcaaagg ctctcacttc ttggctgatg tcctgtggca gattaacaaa
781 acagtagttg gaaattttgg tgaagcaaga attcaagaag aggaagggtcg aaatgaaagt
841 tccagcaatg acatggattg ttaacctca gtgttaagga taactggtgt gacagaaaag
901 gacctgtccc tggaatatga ctgtctggcc ctgaaccttc atggcatgat aaggcacacc
961 ataaggctga gaaggaaaca accaagtaag gagtgtccct cacacattgc t

FIG. 4C

MIDRQRMGLWALAILTLPMYLTVTEGSKSSWGLENEALIVRCPQGRSTYPVEWYYSD
TNESIPTQKRNRIFVSRDRLKFLPARVEDSGIYACVIRSPNLNKTGYLNVTIHKPPSCNIP
DYLMYSTVRGSDKNFKITCPTIDLYNWTAPVQWFKNCKALQEPRFRAHRSYLFIDNVTH
DDEGDYTCQFTHAENGTYIYVATRSFTVEEKGFSMFPVITNPPYNHTMEVEIGKPASIA
CSACFGKGSHFLADVWLQINKTVVGNFGEARIQEEGRNESSNDMDCLTSVLRTGVT
EKDLSLEYDCLALNLHGMIRHTIRLRKQPSKECPSHIA

FIG. 4D

6 / 47

ATGATTGACA	GACAGAGAAT	GGGACTTTGG	GCTTTGGCAA	TTCTGACACT	TCCCATGTAT	60
TTGACAGTTA	CGGAGGGCAG	TAAATCGTCC	TGGGGTCTGG	AAAATGAGGC	TTTAATTGTG	120
AGATGCCCCC	AAAGAGGACG	CTCGACTTAT	CCTGTGGAAT	GGTATTACTC	AGATACAAAT	180
GAAAGTATTC	CTACTCAAAA	AAGAAATCGG	ATCTTTGTCT	CAAGAGATCG	TCTGAAGTTT	240
CTACCAGCCA	GAGTGGAAGA	CTCTGGGATT	TATGCTTGTG	TTATCAGAAG	CCCCAACTTG	300
AATAAGACTG	GATACTTGAA	TGTCACCATA	CATAAAAAGC	CGCCAAGCTG	CAATATCCCT	360
GATTATTTGA	TGTACTCGAC	AGTACGTGGA	TCAGATAAAA	ATTCAAGAT	AACGTGTCCA	420
ACAATTGACC	TGTATAATTG	GACAGCACCT	GTTCAGTGGT	TTAAGAACTG	CAAAGCTCTC	480
CAAGAGCCAA	GGTTCAGGGC	ACACAGGTCC	TACTTGTTC	TTGACAACGT	GACTCATGAT	540
GATGAAGGTG	ACTACACTTG	TCAATTCACA	CACGCGGAGA	ATGGAACCAA	CTACATCGTG	600
ACGGCCACCA	GATCATTAC	AGTTGAAGAA	AAAGGCTTTT	CTATGTTTCC	AGTAATTACA	660
AATCCTCCAT	ACAACCACAC	AATGGAAGTG	GAAATAGGAA	AACCAGCAAG	TATTGCCTGT	720
TCAGCTTGCT	TTGGCAAAGG	CTCTCACTTC	TTGGCTGATG	TCCTGTGGCA	GATTAACAAA	780
ACAGTAGTTG	GAAATTTTGG	TGAAGCAAGA	ATTCAAGAAG	AGGAAGGTCG	AAATGAAAGT	840
TCCAGCAATG	ACATGGATTG	TTTAACCTCA	GTGTTAAGGA	TAACTGGTGT	GACAGAAAAG	900
GACCTGTCCC	TGGAATATGA	CTGTCTGGCC	CTGAACCTTC	ATGGCATGAT	AAGGCACACC	960
ATAAGGCTGA	GAAGGAAACA	ACCAATTGAT	CACCGAAGCA	TCTACTACAT	AGTTGCTGGA	1020
TGTAGTTTAT	TGCTAATGTT	TATCAATGTC	TTGGTGATAG	TCTTAAAAGT	GTTCTGGATT	1080
GAGGTTGCTC	TGTTCTGGAG	AGATATAGTG	ACACCTTACA	AAACCCGGAA	CGATGGCAAG	1140
CTCTACGATG	CGTACATCAT	TTACCCTCGG	GTCTTCCGGG	GCAGCGCGGC	GGGAACCCAC	1200
TCTGTGGAGT	ACTTTGTTCA	CCACACTCTG	CCCGACGTTT	TTGAAAATAA	ATGTGGCTAC	1260
AAATTGTGCA	TTTATGGGAG	AGACCTGTTA	CCTGGGCAAG	ATGCAGCCAC	CGTGGTGGAA	1320
AGCAGTATCC	AGAATAGCAG	AAGACAGGTG	TTTGTTCTGG	CCCCTCACAT	GATGCACAGC	1380
AAGGAATTTG	CCTACGAGCA	GGAGATTGCT	CTGCACAGCG	CCCTCATCCA	GAACAACCTCC	1440
AAGGTGATTC	TTATTGAAAT	GGAGCCTCTG	GGTGAGGCAA	GCCGACTACA	GGTTGGGGAC	1500
CTGCAAGATT	CTCTCCAGCA	TCTTGTGAAA	ATTCAGGGGA	CCATCAAGTG	GAGGGGAAGAT	1560
CATGTGGCCG	ACAAGCAGTC	TCTAAGTTCC	AAATTCTGGA	AGCATGTGAG	GTACCAAATG	1620
CCAGTGCCAG	AAAGAGCCTC	CAAGACGGCA	TCTGTTGCGG	CTCCGTTGAG	TGGCAAGGCA	1680
TGCTTAGACC	TGAAACACTT	TTGA				1704

FIG. 4E

MIDRQRMGLWALAILTLPMYLTVTEGSKSSWGLENEALIVRCPQGRSTYPVEWYYSD
TNESIPTQKRNRIFVSRDRLKFLPARVEDSGIYACVIRSPNLNKTGYLNVTIHKKPPSCNIP
DYLMYSTVRGSDKNFKITCPTIDLYNWTAPVQWFKNCKALQEPRFRAHRSYLFIDNVTH
DDEGDYTCQFTHAENGTYIVTATRSFTVEEKGFSPVITNPPYNHTMEVEIGKPASIA
CSACFGKGSHFLADVLWQINKTVVGNFGEARIQEEEGRNESSSNDMDCLTSVLRITGVT
EKDLSLEYDCLALNLHGMIRHTIRLRKQPIDHRSIYYIVAGCSLLLMFINVLVIVLKVFW
IEVALFWRDIVTPYKTRNDGKLYDAYIIYPRVFRGSAAGTHSVEYFVHHTLPDVLENKC
GYKLCIYGRDLLPGQDAATVVESSIONSRQVFLAPHMMHSKEFAYEQEIALHSALIQ
NNSKVILIEMEPLGEASRLQVGDQLQSLQHLVKIQGTIKWREDHVADKQSLSSKFWKHV
RYQMPVPERASKTASVAAPLSGKACLDLKHf

FIG. 4F

8 / 47

1 atctcaacaa cgagttacca atactgtctc ttgattgata aacagaatgg ggttttggat
 61 cttagcaatt ctacaaatc tcatgtattc cacagcagca aagtttagta aacaatcatg
 121 gggcctggaa aatgaggctt taattgtaag atgtcctaga caaggaaaac ctagtacac
 181 cgtggattgg tattactcac aaacaaacaa aagtattccc actcaggaaa gaaatcgtgt
 241 gtttcctca ggccaacttc tgaagtttct accagctgaa gttgctgatt ctggtattta
 301 tacctgtatt gtcagaagtc ccacattcaa taggactgga tatgcgaatg tcaccatata
 361 taaaaaacia tcagattgca atgtccaga ttattgatg tattcaacag tatctggatc
 421 agaaaaaaat tccaaaattt attgtcctac cattgacctc tacaactgga cagcaccctc
 481 tgagtgggtt aagaattgtc aggtcttca aggatcaagg tacaggggcg acaagtcatt
 541 tttgctcatt gataatgtga tgactgagga cgcaggtgat tacacctgta aatttatata
 601 caatgaaaat ggagccaatt atagtgtgac ggcgaccagg tccttcacgg tcaaggatga
 661 gcaaggcttt tctctgttc cagtaatcgg agcccctgca caaatgaaa taaaggaagt
 721 ggaaattgga aaaaacgcaa acctaacttg ctcgtctgt ttggaaaag gcactcagtt
 781 cttggctgcc gtctgtggc agcttaattg acaaaaaatt acagacttg gigaaccaag
 841 aattcaacaa gaggaagggc aaaatcaaag tttagcaat gggctggctt gtctagacat
 901 ggttttaaga atagctgacg tgaaggaaga ggatttattg ctgcagtacg actgtctggc
 961 cctgaatttg catggcttga gaaggcacac cgtaagacta agtaggaaaa atccaagtaa
 1021 ggagtgttgc tgagacttg atcacctgaa ctttcttag caagtgtag cagaatggag
 1081 tgtggtcca agagatccat caagacaatg ggaatggcct gtgccataaa atgtgcttct
 1141 cttctcggg atgtgtttg ctgtctgac ttttagact gttcctgtt gctgggagct
 1201 tctctgtgc ttaaattgt cgtctcccc cactccctcc tctgttgg ttgtctagaa
 1261 cactcagctg cttcttgggt catcttgtt ttctaactt atgaactccc tctgtgtcac
 1321 tgtatgtgaa aggaaatgca ccaacaaccg aaaactg

FIG. 4G

MGFWILAILTILMYSTAAKFSKQSWGLENEALIVRCPRQGKPSYTVDWYYSQTNKSIPT
 QERNRVFASGQLLKFLPAEVADSGIYTCIVRSPTFNRTGYANVTIYKKQSDCNVPDYL
 YSTVSGSEKNSKIYCPTIDLYNWTALEWFKNCQALQGSRYRAHKSFLVIDNVMTEAG
 DYTCKFIHNENGANYSVTATRSFTVKDEQGFSLFPVIGAPAQNEIKEVEIGKNANLTCSA
 CFGKGTQFLAAVLWQLNGTKITDFGEPRIQQEQQNQSFNGLACLDMLVRIADVKEED
 LLLQYDCLALNLHGLRRHTVRLSRKNPSKECF

FIG. 4H

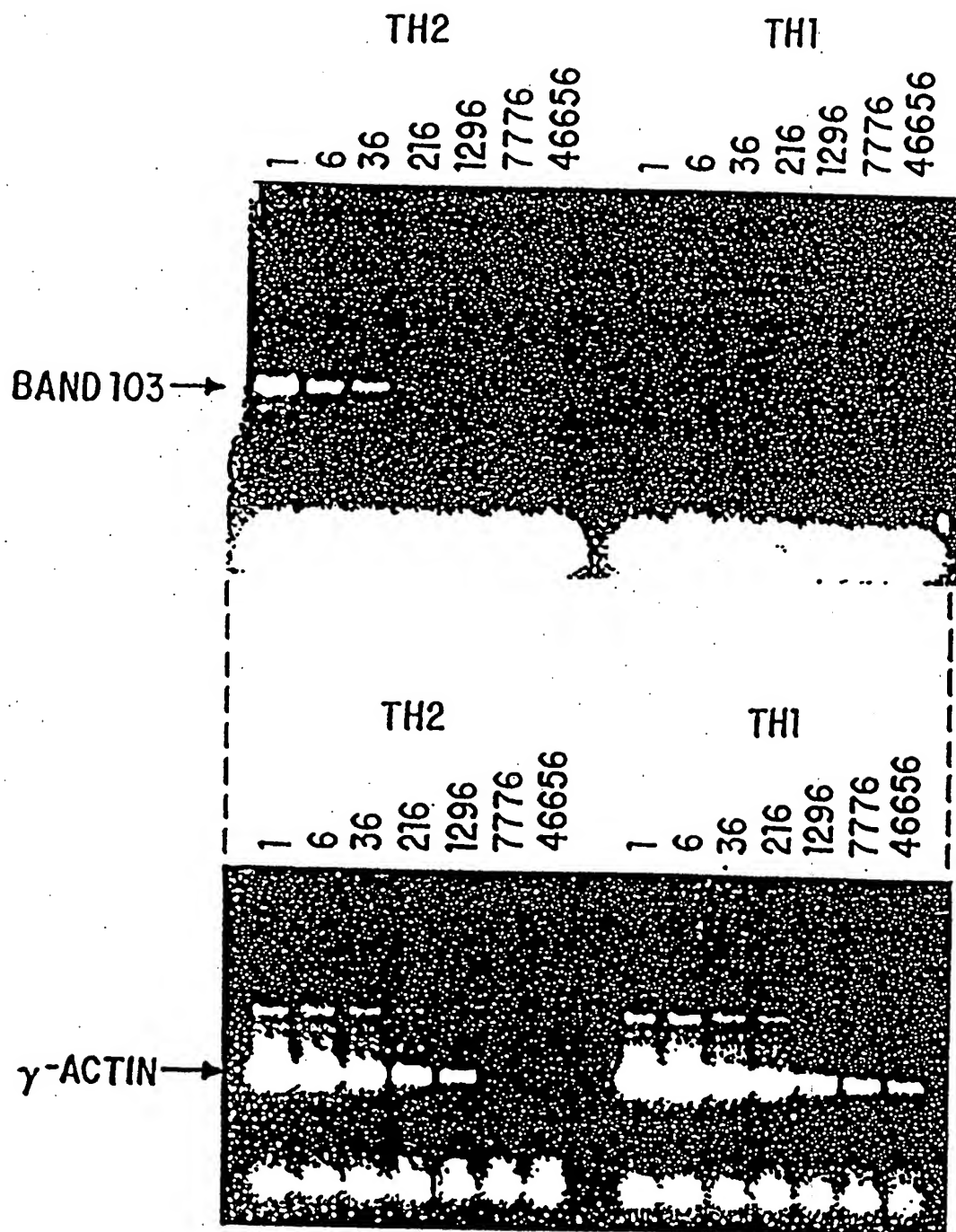


FIG. 5

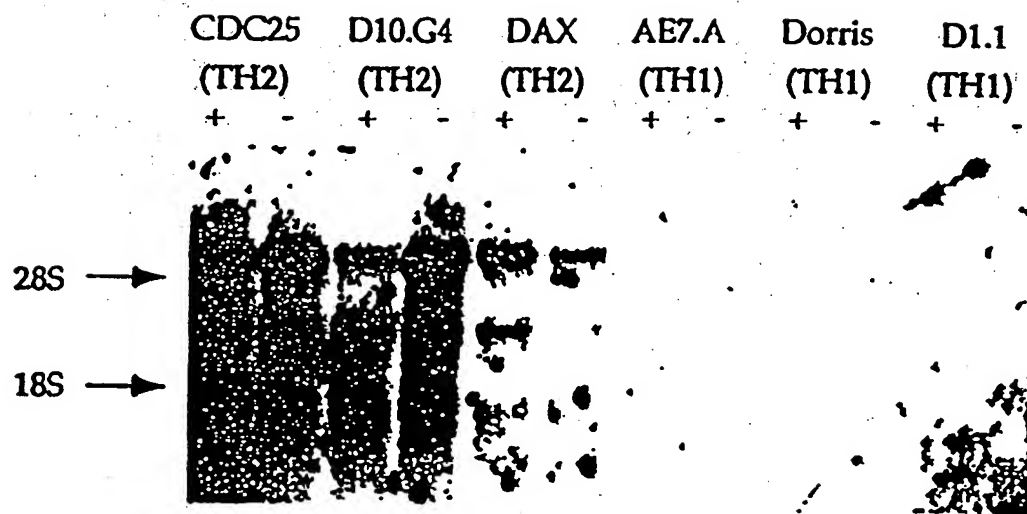


FIG. 6

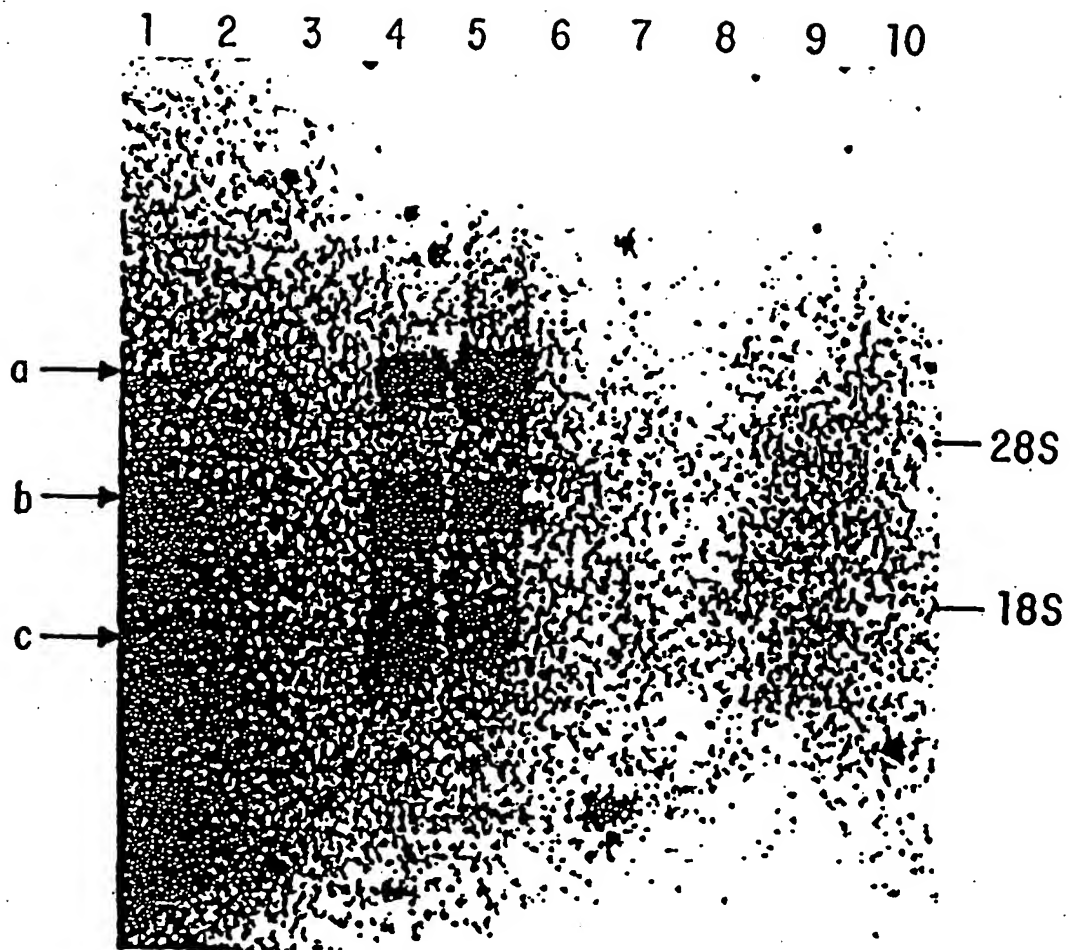


FIG. 7

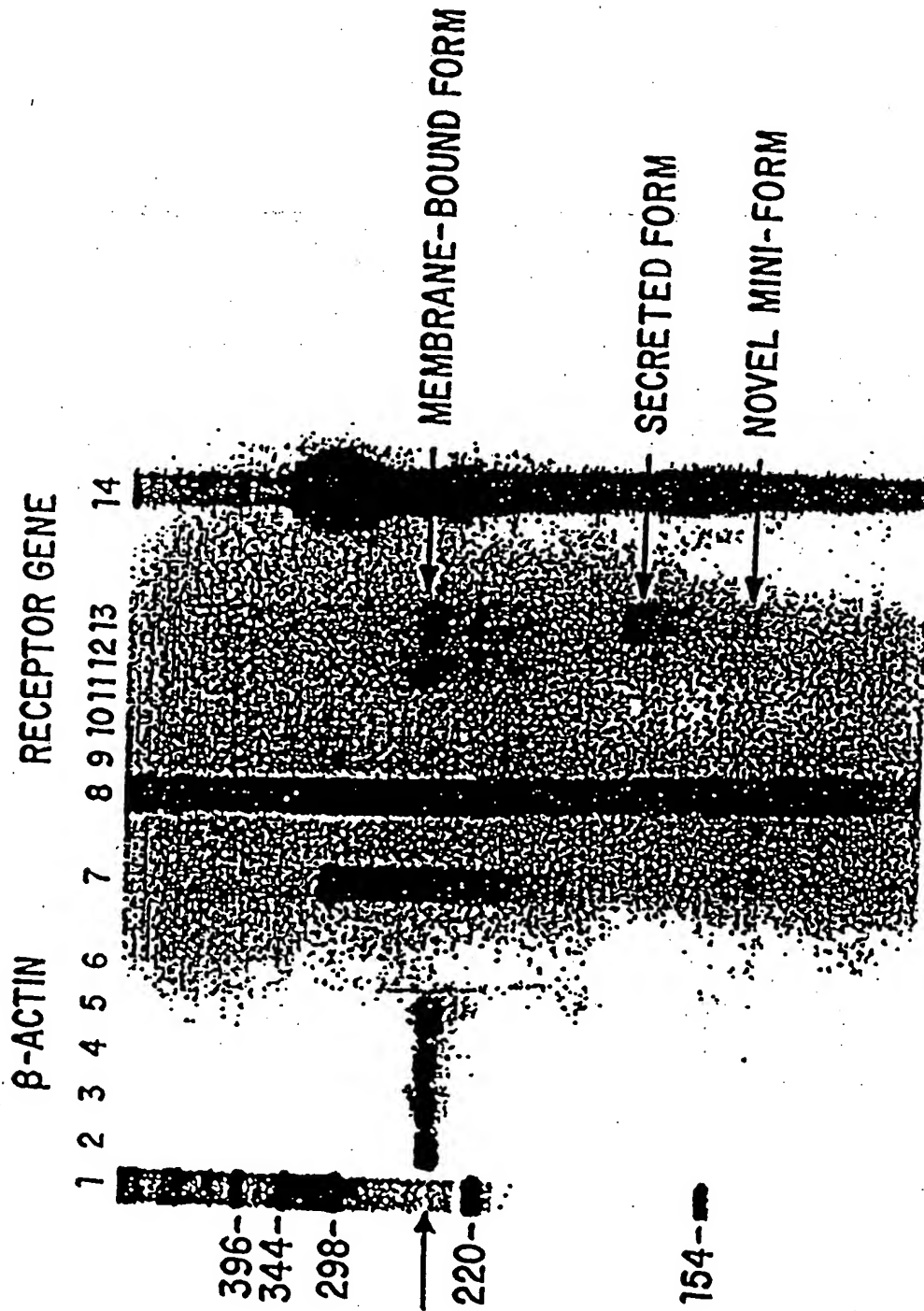


FIG. 8

	CC	2
GGGTCGACCCACGCGTCCGAGCCTCCTCAGTCAAGAGAAGCATCCCTCCAGAAACAGGAAACATGACACTTTTGAAAG		81
AATGCCAAACGGCGTGAAAAATAAAACAGAGCATTCCCATTTGCACCGACCAATCTCCAATCTCCTGTAGATTCAAAA		160
GGGCAAGCAAGAGCGGTGACCGTTACGAAAGCTAAATCCCATGCTATTGAACATGAAGACTTCTGATGCTTAAATC		239
TCATTAACTGCTTTAAGTCACTCCAGGAGCTTGGATCCCAACTTCTAGCAGTAATAGTCTGTGTAAAAAATAAAAAA		318
AATCAGTCTACAACCACTCTCTAAATGCATGGATGAACCTCATCAGAACATCAAAACCCCAAGGAAACCCTAAGAGAGAAG		397
AATTCTAATAAAAGAATTTTACATTGAAAACTTACAAGGCAAGTCCCTTTCCCTGCTGACAGCCTAAGAAAGTGATGT		476
	M A M N S M C I E E Q R H L E	15
AACTGCCACTGTGAAGACC ATG GCG ATG AAC AGC ATG TGC ATT GAA GAG CAG CGC CAC CTC GAA		540
H Y L F P V V Y I I V F I V S V P A N I		35
CAC TAT TTG TTC CCG GTG GTC TAC ATA ATT GTG TTT ATA GTC AGC GTC CCA GCC AAC ATC		600
G S L C V S F L Q A K K E N E L G I Y L		55
GGA TCT TTA TGC GTA TCC TTT CTG CAA GCG AAG AAG GAA AAT GAG CTA GGG ATT TAC CTC		660
F S L S L S D L L Y A L T L P L W I N Y		75
TTC AGT CTG TCC CTG GAC CTG CTG TAT GCG CTG ACT CTG CCC CTC TGG ATC AAT TAC		720

FIG. 9A

T W N K D N W T F S P T L C K G S V F F 95
 ACT TGG AAT AAA GAC AAC TGG ACT TTC TCT CCC ACC TTG TGC AAA GGA AGC GTT TTC TTC 780

T Y M N F Y S S T A F L T C I A L D R Y 115
 ACC TAC ATG AAC TTT TAC AGC AGC AGC GCG TTC CTC ACT TGC ATT GCC CTG GAC CGC TAT 840

L A V V Y P L K F S F L R T R R F A F I 135
 TTA GCA GTC GTC TAC CCT CTG AAG TTT TCC TTC CTA AGA ACG AGA AGA TTC GCG TTT ATT 900

T S L S I W I L E S F F N S M L L W K D 155
 ACC AGC CTC TCC ATC TGG ATA TTA GAG TCC TTC TTT AAC TCT ATG CTT CTG TGG AAA GAT 960

E T S V E Y C D S D K S N F T L C Y D K 175
 GAA ACG AGT GTT GAA TAT TGT GAC TCG GAC AAA TCT AAT TTC ACT CTC TGC TAT GAC AAA 1020

Y P L E K W Q I N L N L F R T C M G Y A 195
 TAC CCT CTG GAG AAA TGG CAG ATA AAC CTC AAC CTG TTT CGG ACG TGC ATG GGC TAC GCA 1080

I P L I T I M I C N H K V Y R A V R H N 215
 ATA CCC TTG ATC ACC ATC ATG ATC IGC AAC CAT AAA GTC TAC CGA GCT GTG CGG CAC AAC 1140

Q A T E N S E K R R I I K L L A S I T L 235
 CAA GCC ACG GAA AAC AGC GAG AAG AGA AGG ATC ATA AAG TTG CTT GCT AGC ATC ACG TTG 1200

T F V L C F T P F H V M V L I R C V L E 255
 ACT TTC GTC CTA TGC TTT ACC CCC TTC CAC GTG ATG GTG CTC ATC CGC TGC GTT TTA GAG 1260

FIG. 9B

15 / 47

R D M N V N D K S G W Q T F T V Y R V T 275
CGC GAC ATG AAC GTC AAT GAC AAG TCT GGA TGG CAG ACG TTT ACG GTG TAC AGA GTC ACA 1320

V A L T S L N C V A D P I L Y C F V T E 295
GTA GCC CTG ACG AGT CTA AAC TGT GTT GCC GAT CCC ATT CIG TAC TGC TTT GTG ACT GAG 1380

T G R A D M W N I L K L C T R K H N R H 315
ACG GGG AGA GCT GAT ATG TGG AAC ATA TTA AAA TTG TGT ACT AGG AAA CAC AAT AGA CAC 1440

Q G K K R D I L S V S T R D A V E L E I 335
CAA GGG AAA AAA AGG GAC ATA CTT TCT GTG TCC ACA AGA GAT GCT GTA GAA TTA GAG ATT 1500

I D * 338
ATA GAC TAA GAGGTGGAGGCAGGTTAAGTTACATGGTATTATTTAATGAACTTACATTTTGGAAAGAAATCTGG 1576

CATAGTAGAACCCAGTGGAATAGTTGAAGGTACATTGTATGACTCCTATGTGGCTTTATTAAGTAAGGTATAGAAA 1655

TGTATTATCTTGATGTATTCTAATGACTAGGCATCATTTGTTTAGTACCAATTCTCTTGGCCTCTATGTTATAACCCC 1734

TAAGAAGCAGCGGGACTGTTGCTCTTAAATCAGTGGCCATTCTATCTGACTACTATGACTTTTGTGTTGTTCTGC 1813

TTTGGGTTTCAGTCTGCCTGCATCAGTCTTCTCCTCTGTATACGTCTGTCTTCAACAAATGTAAGGACTAAATACCCC 1892

FIG. 9C

TCCCGATCACATCCATTATCAAGGATTTGAAGCCACTCCCATGTACTGGGTTATAAAGAAATGTTCTCATGAACTTTCA 1971
TGAAGTTTACATACCTTTGGGGATCTAGTCACCGAGTCACATAAAGTAAATGGAAAAAATAAAAAA 2050
AGGGC 2055

FIG. 9D

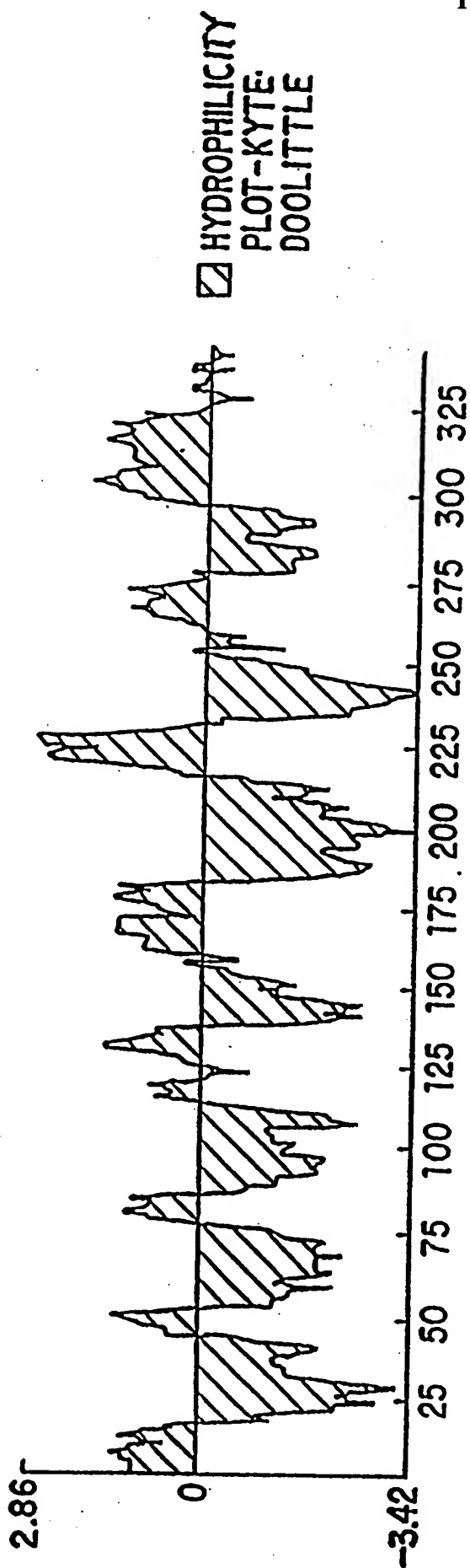


FIG. 10A

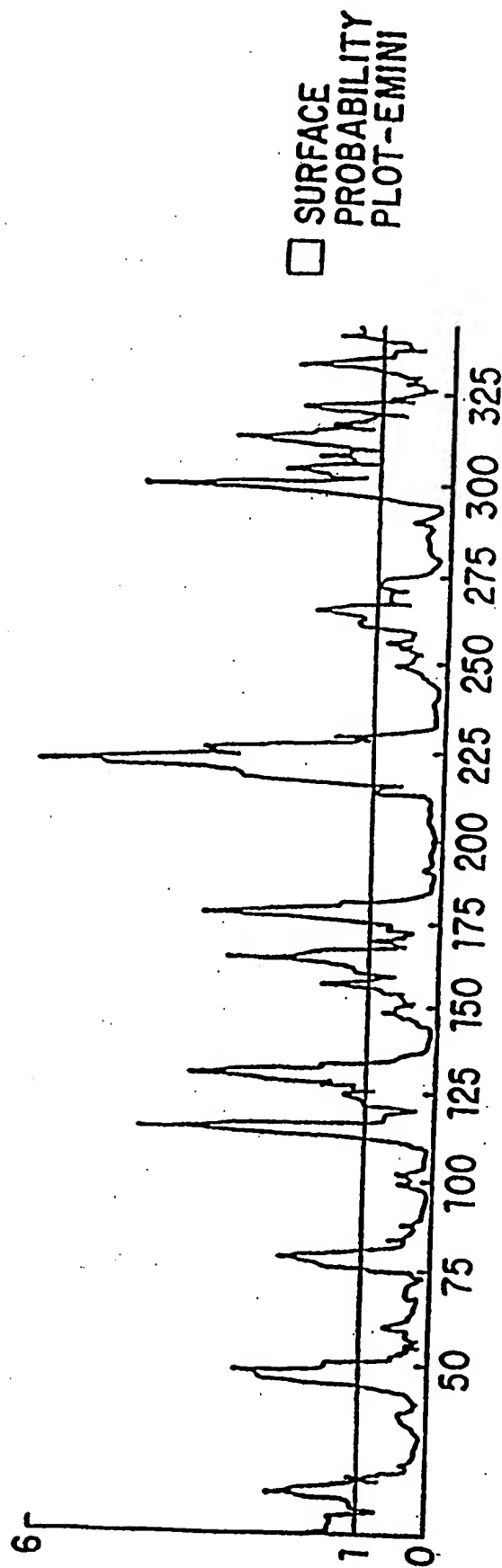


FIG. 10B

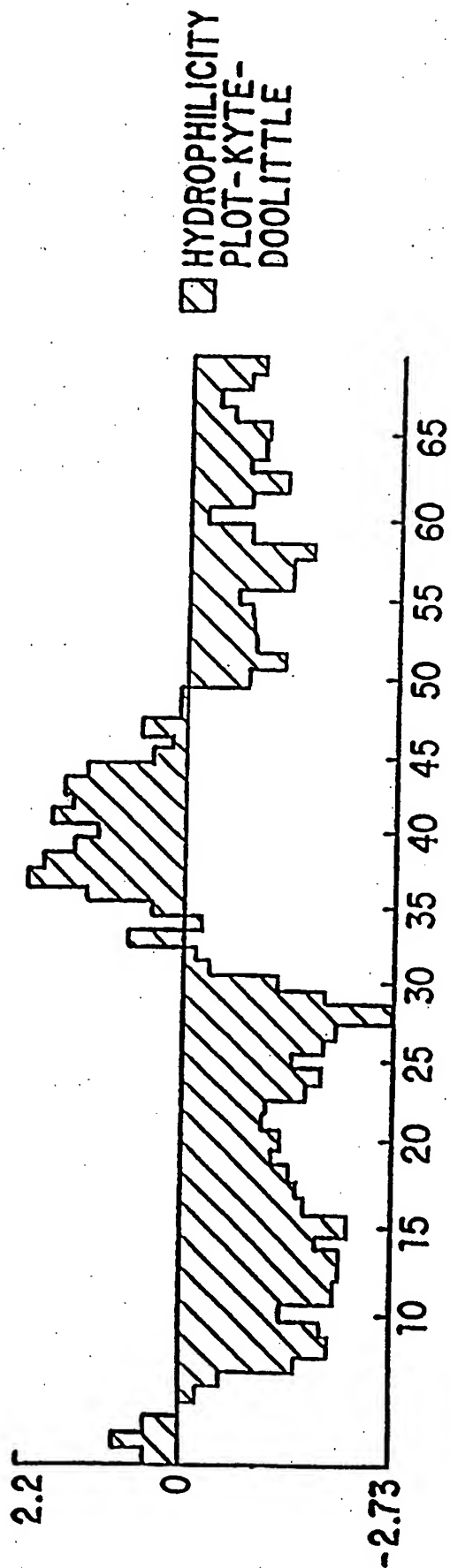


FIG. 10C

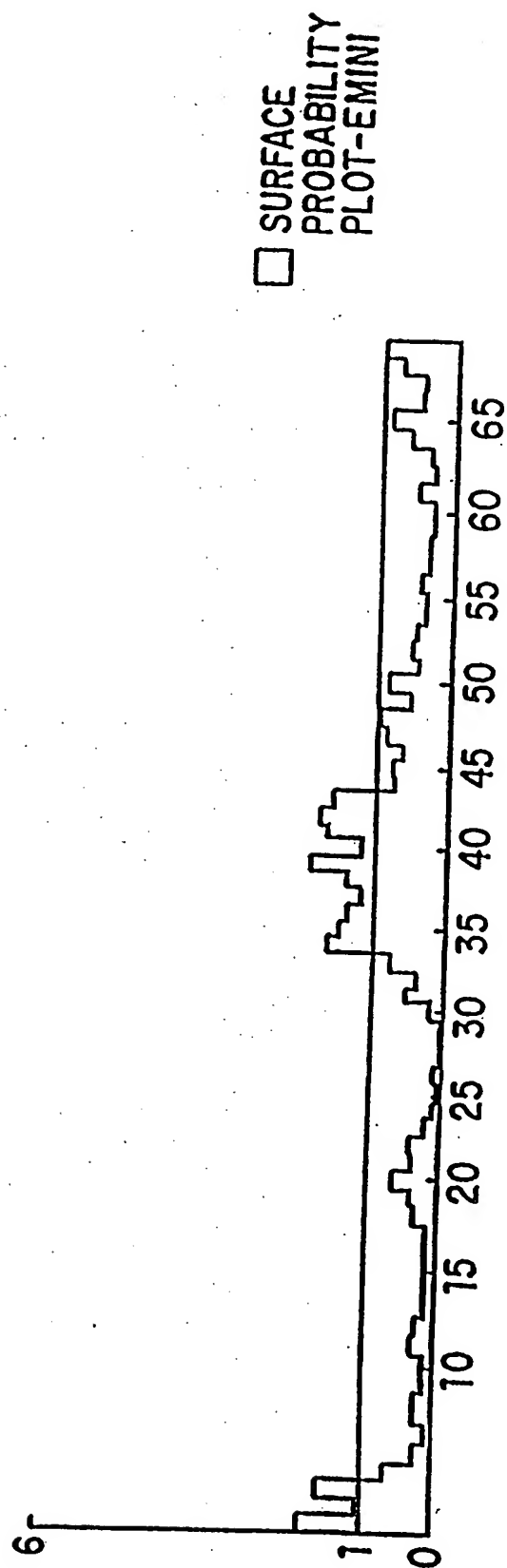


FIG. 10D

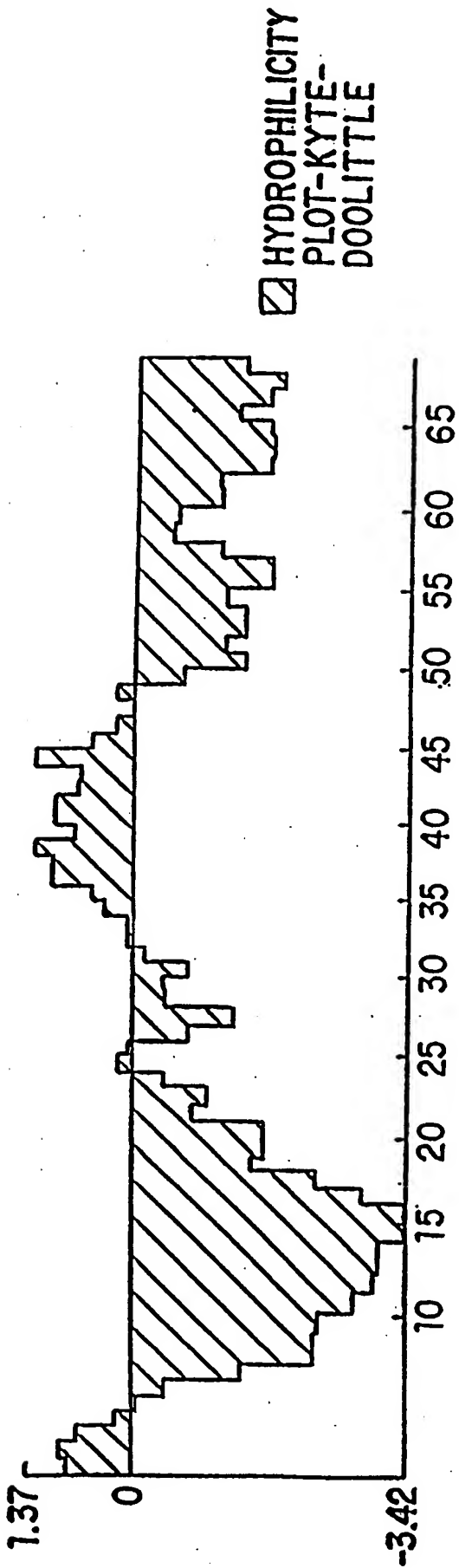


FIG. 10E

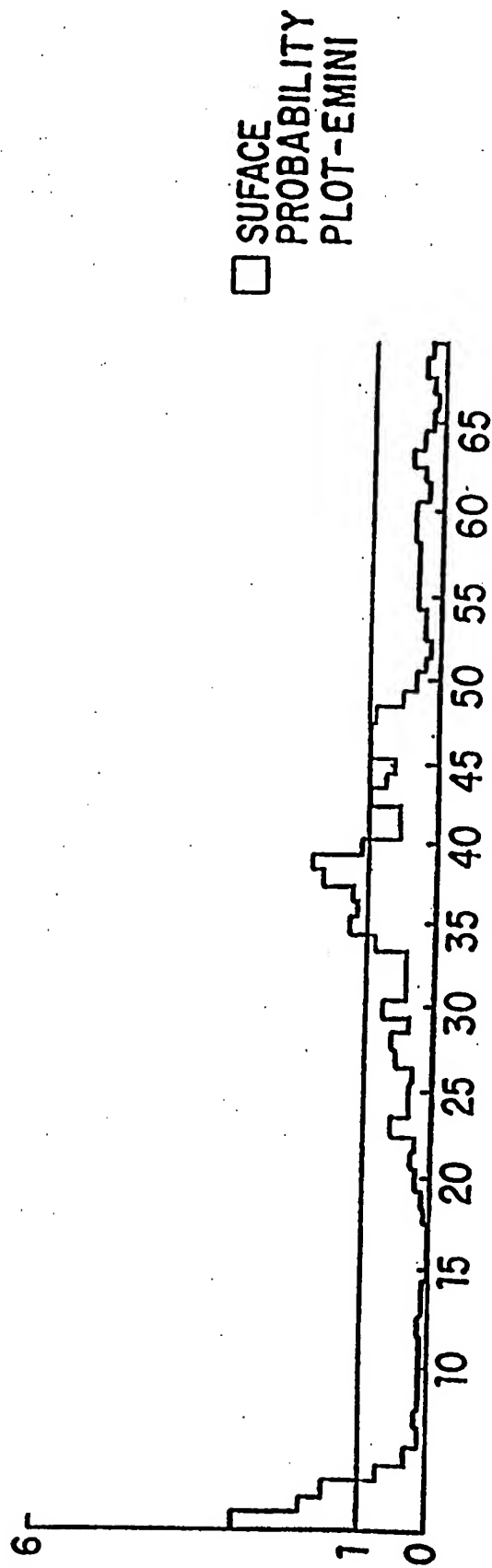
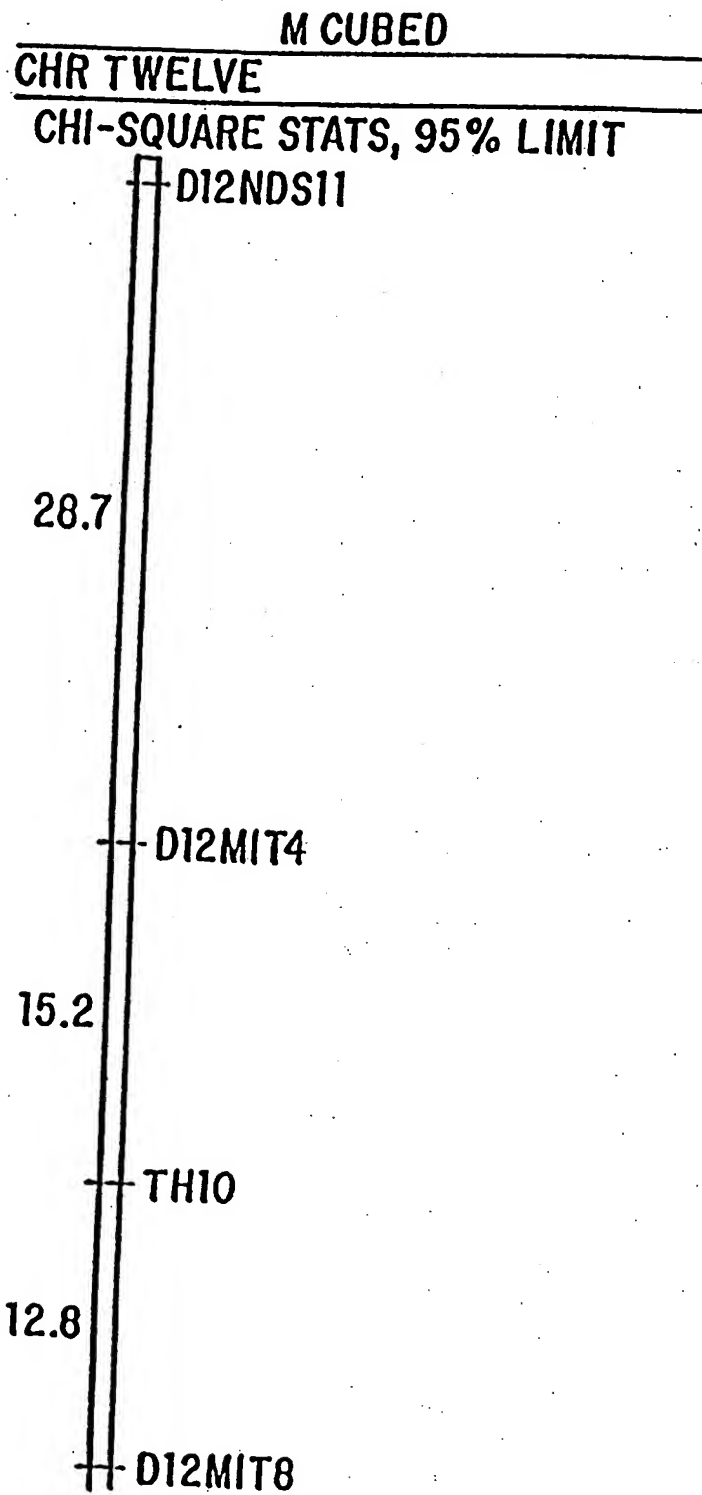


FIG. 10F

20 / 47

**FIG. 11**

21 / 47

CGCCAGTGTGCTGGAATTCGGCTTAGAGCATTTCCTTCA
AACCACAGGTTACACACACTTACTAAAAGCAATGCTG
TTAGAGGAGAAGGGCTTGGGAGACTCGGCCATTTGAAAC
ANAAGCAAGGCACTCTCCAGGNNCAGCAAGTGGATTCCC
ATTTCTGCTGAGGGCGGGTTCACACTGAGACTGCACTC
CAGTCAGCGGGAGGAATCACCTGCATTAATGCTTGTCT
CTGCAGAGCTAGTGTGCCTTCCACTCTGGGTACACTTGG
GTGTCAACATTTCAAAATGATGACCTAAGAGGCTCTCAT
AGTTGGTGATAACTATGGNAGGACAGMGMCACTGGCT
GTATTGTCTTTTCTTTCAGCACTAGTGTCTTGGCCCTT
AATAAACGGGTTCATCATCTCCAAACCAGGAGAT
AGATTGTTAGACAGGTCCTTTCCCCTCAACT

FIG. 12

TTTNNGGGACAGGGTTTCNCTGTGTATCTCTGGCTGTCC
TGGAACNACTCTGTAGACCAGGTTGGCCTCGANCTCAG
AAATCTACCTGCCTCTCCCTCCANAGTGCTGGGATTAAN
GGTGTATGCCACCAATNCCCGGCCTTAATATATTNNTAA
ACAACTTCATTTGAATGANATATTGACACTACCTTGGA
ATAAGAGTNCCCAGAATGANGTACAGGNTTCANGGAATC
ATTAA

FIG. 14

CTTAGCAGGTGGAGTTGCAGCAGGAAGCCTGGTAGCCAC
ACTCCAATCAGCAGGGTCCCTTGGACTCTCCACATCAAC
AAATGCCATCCTAGGGGCTGCTGGGGCACTGTTGGAGCC
TTGCTCTGAGCTTAGGAGATGACACTTCTATCAGCTCA
CTCAAAGCCTGTACAGACTACGCAGGAGATGAAGTTCCA
AAAGGCACCTTCAGAACCTCA

FIG. 15

10 TTTTTTTT 20 TNGGAGAGG CTAGCACTGA AATTACAGTT TCAGTGGAA 50 TTAGAGAAGT AATAACTGCA 70
AAAATTTATT TACACACACA CACACACACA CAGGGCATT TACCTGTGTA AGTGCAGTTT AATCANCCCC 140
ATTACCTTAT GACCTTGGT GGCAATGTCT CTAAAGCTT AAAATTAAAA TAAATTTAA AAGATGGTTT 210
TCCATCTCAT AAAATCCCCT TTGGGAATGG AAGACTTCCT CTTTGGGGTN TTTTATTAGAG GGAACAGGAG 280
GTAACGTGTA ATTATTTATA CATTCTAATA AACCATGAAT GCACCACATA AAATACTGTA CTCGGGGAGC 350
AAACACTGTN TGGGGGGGTT CTCTCTTACC AGAAGGAACA GGGGGCTTTT CAATGGCTGT GGGC 414

FIG. 13

remt161g0f	F-----		BAND 161
g1/218574/	MRQKAVSLFLCYLLLFTCSGVEAGKKCESDSDSGSF-WKALTFMAVGGGLAVAGLP--	CHIMP GENE	
g1/32698/g	MRQKAVSVFLCYLLLFTCSGVEAGKKCESDSDSGSF-WKALTFMAVGGGLAVAGLP--	HUMAN 6-16	
g1/32701/g	-----VEAGKKCESDSDSGSF-WKALTFMAVGGGLAVAGLP--	HUMAN 6-16	
g1/32702/g	-----GKKKCESDSDSGSF-WKALTFMAVGGGLAVAGLP--	HUMAN 6-16	
g1/35184/g	MEASAL-----TSSAVTSVAKWVRVASGSAAVLPLARIATTVIGGVWAMAAPMV	HUMAN P27	
remt161g0f	---FVFLA-----GGVAAGSLVATLQSAGVLGLSTSTNAILGAA	BAND 161	
g1/218574/	--ALGFTGAGIAANSVAASLSMSAISAILNGGGVPAGGLVATLQSLGAGG----	CHIMP GENE	
g1/32698/g	--ALGFTGAGIAANSVAASLSMSAISAILNGGGVPAGGLVATLQSLGAGG----	HUMAN 6-16	
g1/32701/g	--ALGFTGAGIAANSVAASLSMSAISAILNGGGVPAGGLVATLQSLGAGG----	HUMAN 6-16	
g1/32702/g	--ALGFTGAGIAANSVAASLSMSAISAILNGGGVPAGGLVATLQSLGAGG----	HUMAN 6-16	
g1/35184/g	LSAMGFTAAGIASSIIAAKMMSAAAANGGGVASGSLVGTLQSLGATGLSGLTKFILGSI	HUMAN P27	
remt161g0f	GALLEPCSELRR-----	BAND 161	
g1/218574/	GALMGYATHKYLDSEEDDEE	CHIMP GENE	
g1/32698/g	GALMRYATHKYLDSEEDDEE	HUMAN 6-16	
g1/32701/g	GALMRYATHKYLDSEEDDEE	HUMAN 6-16	
g1/32702/g	GALMRYATHKYLDSEEDDEE	HUMAN 6-16	
g1/35184/g	GSAIAAVIARFY	HUMAN P27	

FIG. 16

```

      M   F   S   G   L   T   L   6
NGTCGACCCAGCGGTCCGGATTTCCTCCCAAGTACTC ATG TTT TCA GGT CTT ACC CTC 60

N   C   V   L   L   L   L   Q   L   L   L   A   R   S   L   E   D   G   Y   K   26
AAC TGT GTC CTG CTG CAA CTA CTA CTT GCA AGG TCA TTG GAA GAT GGT TAT AAG 120

V   E   V   G   K   N   A   Y   L   P   C   S   Y   T   L   P   T   S   G   T   46
GTT GAG GTT GGT AAA AAT GCC TAT CTG CCC TGC AGT TAC ACT CTA CCT ACA TCT GGG ACA 180

L   V   P   M   C   W   G   K   G   F   C   P   W   S   Q   C   T   N   E   L   66
CTT GTG CCT ATG TGC TGG GGC AAG GGA TTC TGT CCT TGG TCA CAG TGT ACC AAT GAG TTG 240

L   R   T   D   E   R   N   V   T   Y   Q   K   S   S   R   Y   Q   L   K   G   86
CTC AGA ACT GAT GAA AGA AAT GTG ACA TAT CAG AAA TCC AGC AGA TAC CAG CTA AAG GGC 300

D   L   N   K   G   D   V   S   L   I   I   K   N   V   T   L   D   D   H   G   106
GAT CTC AAC AAA GGA GAT GTG TCT CTG ATC ATA AAG AAT GTG ACT CTG GAT GAC CAT GGG 360

T   Y   C   C   R   I   Q   F   P   G   L   M   N   D   K   K   L   E   L   K   126
ACC TAC TGC TGC AGG ATA CAG TTC CCT GGT CTT ATG AAT GAT AAA AAA TTA GAA CTG AAA 420

L   D   I   K   A   A   K   V   T   P   A   Q   T   A   H   G   D   S   T   T   146
TTA GAC ATC AAA GCA GCC AAG GTC ACT CCA GCT CAG ACT GCC CAT GGG GAC TCT ACT ACA 480

A   S   P   R   T   L   T   T   E   R   N   G   S   E   T   Q   T   L   V   T   166
GCT TCT CCA AGA ACC CTA ACC ACG GAG AGA AAT GGT TCA GAG ACA CAG ACA CTG GTG ACC 540

```

FIG. 17A

L H N N N G T K I S T W A D E I K D S G 186
CTC CAT AAT AAC AAT GGA ACA AAA ATT TCC ACA TGG GCT GAT GAA ATT AAG GAC TCT GGA 600

E T I R T A I H I G V G V S A G L T L A 206
GAA ACG ATC AGA ACT GCT ATC CAC ATT GGA GTG GGA GTC TCT GCT GGG TTG ACC CTG GCA 660

L I I G V L I L I L K W Y S C K K K L S S 226
CTT ATC ATT GGT GTC TTA ATC CTT AAA TGG TAT TCC TGT AAG AAA AAG AAG TTA TCG AGT 720

L S L I T L A N L P P G G L A N A G A V 246
TTG AGC CTT ATT ACA CTG GCC AAC TTG CCT CCA GGA GGG TTG GCA AAT GCA GGA GCA GTC 780

R I R S E E N I Y T I E E N V Y E V E N 266
AGG ATT CGC TCT GAG GAA AAT ATC TAC ACC ATC GAG GAG AAC GTA TAT GAA GTG GAG AAT 840

S N E Y Y C Y V N S Q Q P S * 280
TCA AAT GAG TAC TAC TGC TAC GTC AAC AGC CAG CAG CCA TCC TGA CCGCCTCTGGACTGCCACT 903

TTTAAAGGCTCGCCTTCATTTCTGACTTTGGTATTTCCCTTTKTGGAAACTATGTGATATGTCACTTGGCAACCTCAT 982

TGGAGGTTCTGACCACAGCCACTGAGAAAAGAGTTCCAGTTTCTGGGGATAATTAACACAAAGGGGATTCCGACTGTA 1061

ACTCATGCTACATTGAAATGCTCCATTTTATCCCTGAGTTTCAGGGATCGGATCTCCCACTCCAGAGACTTCAATCATG 1140

CGTGTTGAAGCTCACTCGTGCTTTCATACATTAGGAATGGTGTAGTGTGATGCTTTTGAGACATAGAGGTTTGTGGTATA 1219

FIG. 17B

TCCGCAAGCTCCTGAACAGGTAGGGGAATAAAGGGCTAAGATAGGAAGGTGCGGYCTTTGTTGATGTTGGAAAATC 1298
TTAAGAAGTTGGTAGCTTTTCT AGAGATTTCTGACCTTGAAGATTGAAGAAAAGCCAGGTGGCATATGCTTAACAC 1376
GATATAACTTGGGAACCTTAGGCAGGAGGTGATAAGTTCAAGGTCAGCCAGGGCTATGCTGGTAAGACTGTCTCAMCA 1455
TCCAAAGACGAAAAATAACATAGAGACAGCAGGAGGCTGGAGATGAGGCTCGGACAGTGAGGTGCATTGTGTACAAGCA 1534
CGAGGAATCTATATTTGATCGTAGACCCCCACATGAAAAAGCTAGGCCTGGTAGAGCATGCTTGTAGACTCAAGAGATGG 1613
AGAGGTAAGGCACAACAGATCCCCGGGGCTTGCGTGCACTCAGCTTAGCCTAGGTGCTGAGTTCCAAGTCCACAAGAG 1692
TCCCTGTCTCAMAAGTAGGTGAGTATCTGGCGCATGTCCATGGGGGTGTCCCTCTCCTCCTCAGAAAGACATGC 1771
ACATGACCCTGCACACACACACACACACACACACACACACACACATGAAATGAAGGTTCTCTCTG 1850
TGCCTGCTACCTCTATAACATGTATCTCTACAGGACTCTCCTCTGCCTCTGTTAAGACATGAGTGGGAGCATGGCAG 1929
AGCAGTCCAGTAATTTATCCAGCACTCAGAAGGCTGGAGCAGAAGCGTGGAGAGTTCAGGAGCACTGTGCCCCAACACT 2008
GCCAGACTCTTCTTACACAAGAAAAAGGTTACCCGCAAGCAGCCTGCTGTCTGTAAAGGAAACCCCTGCCGAAAGGCAA 2087
CTTTGACTGTTGTGCTCAAGGGAACTGACTCAGACAACCTCTCCATTCTCTGGAGGAACTGGAGCTGTTTCTGACA 2166
GAAGACAACCGGTGACTGGGACATACGAAGGCAGAGCTCTTGACGAATCTATATAGTCAGCAAAATATCTTTGGGA 2245

FIG. 17C

27 / 47

GGACAGTCGTACCAAAATTGATTTCCAAGCCGGTGGACCTCAGTTTCATCTGGCTTACAGCTGCCTGCCCCAGTGCCCTT 2324
GATCTGTGCTGGCTCCCATCTATAACAGAAATCAAAATTAATAGACCCCGAGTGAAAAATTAAGTGAGCAGAAAGGTAG 2403
CTTTGTTCAAAGATTTTTTGCATTGGGGAGCAACTGTGTACATCAGAGGACATCTGTTAGTGAGGACACCAAAACCTG 2482
TGGTACCGTTTTTTCATGTATGAATTTTGTGTTTAGGTTGCTTCTAGCTAGCTGTGGAGTCTGGCTTCTTAGGTG 2561
GGTATGGAAGGGAGACCATCTAACAAAATCCATTAGAGATAACAGCTCTCATGCAGAAGGGAAAACTAATCTCAAATGT 2640
TTTAAAGTAATAAACTGTACTGGCAAAGTACTTTGAGCATAAAAAAGGGGGCGCCGC 2710

FIG. 17D

28 / 47

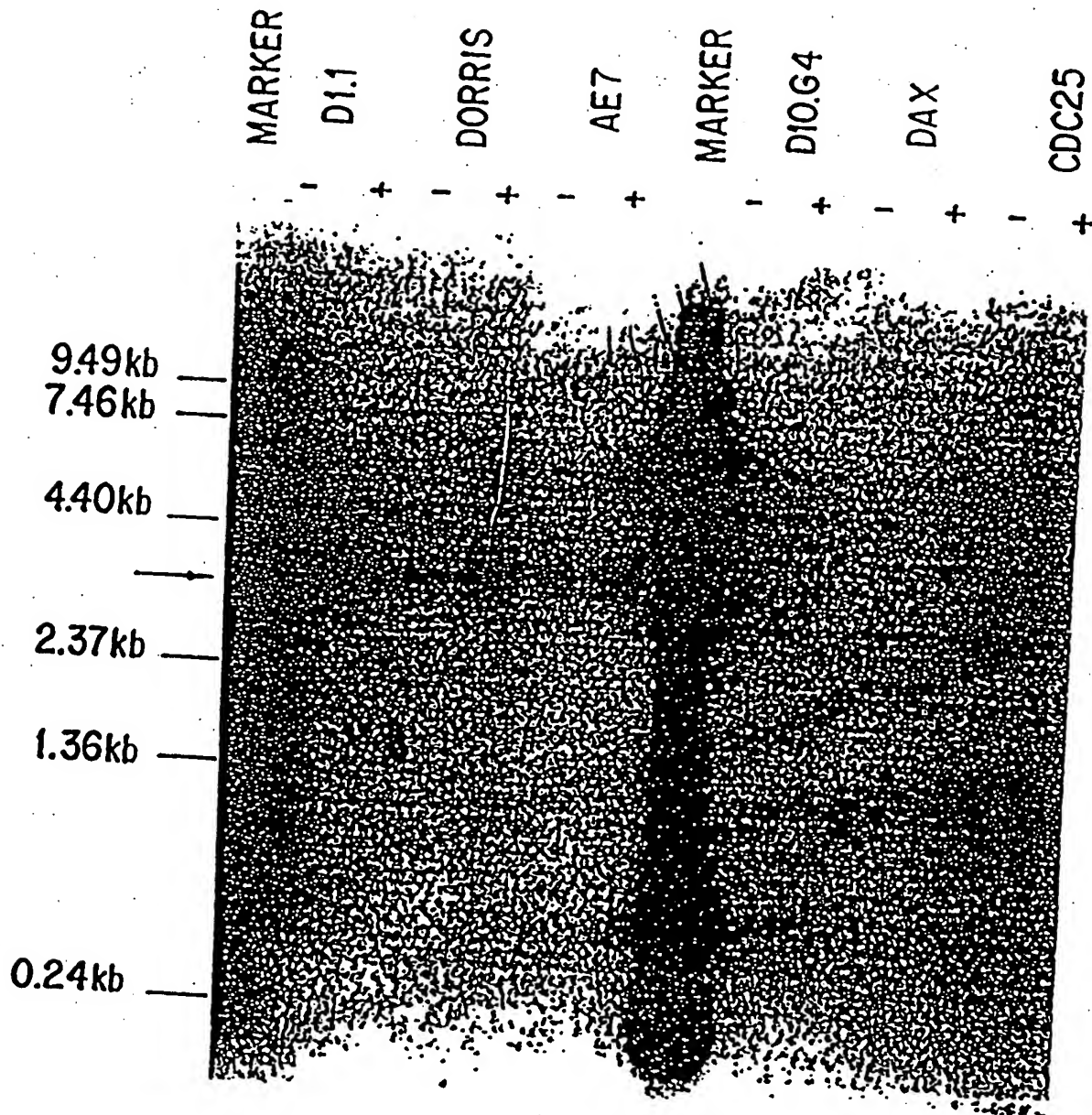


FIG. 18

29 / 47

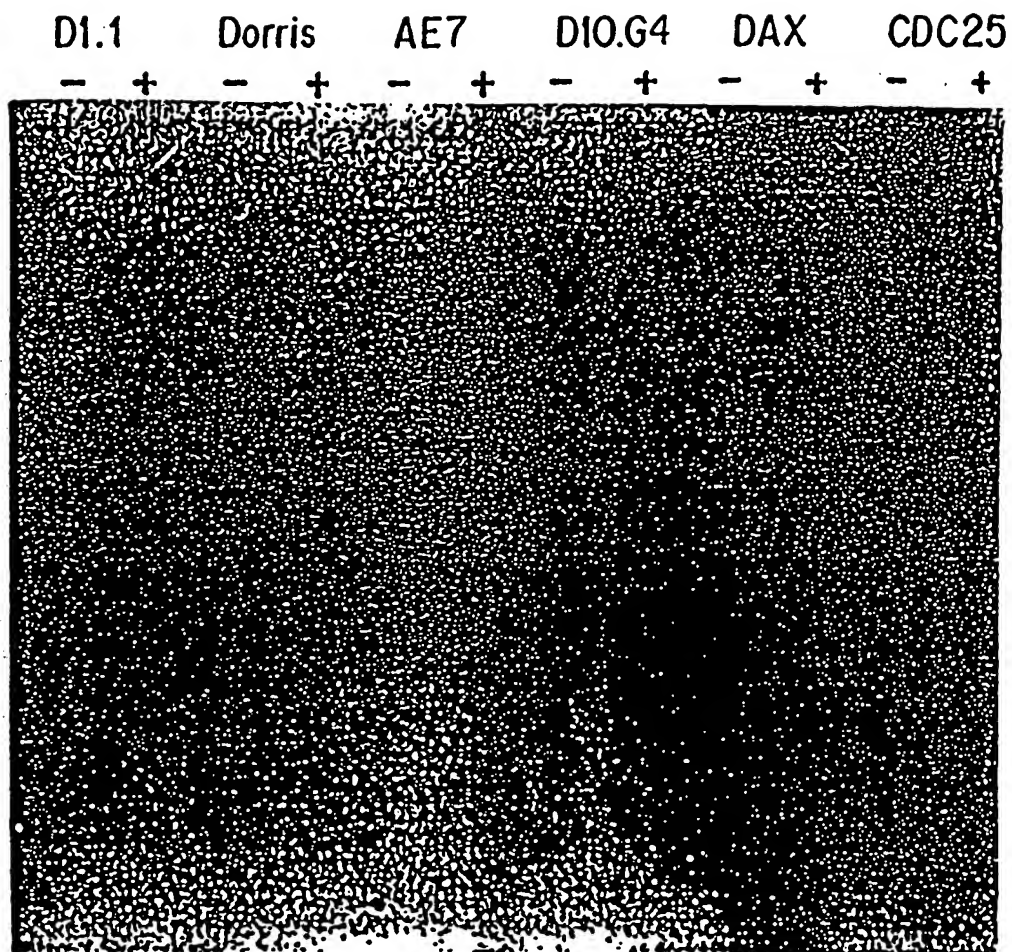


FIG. 19

30 / 47

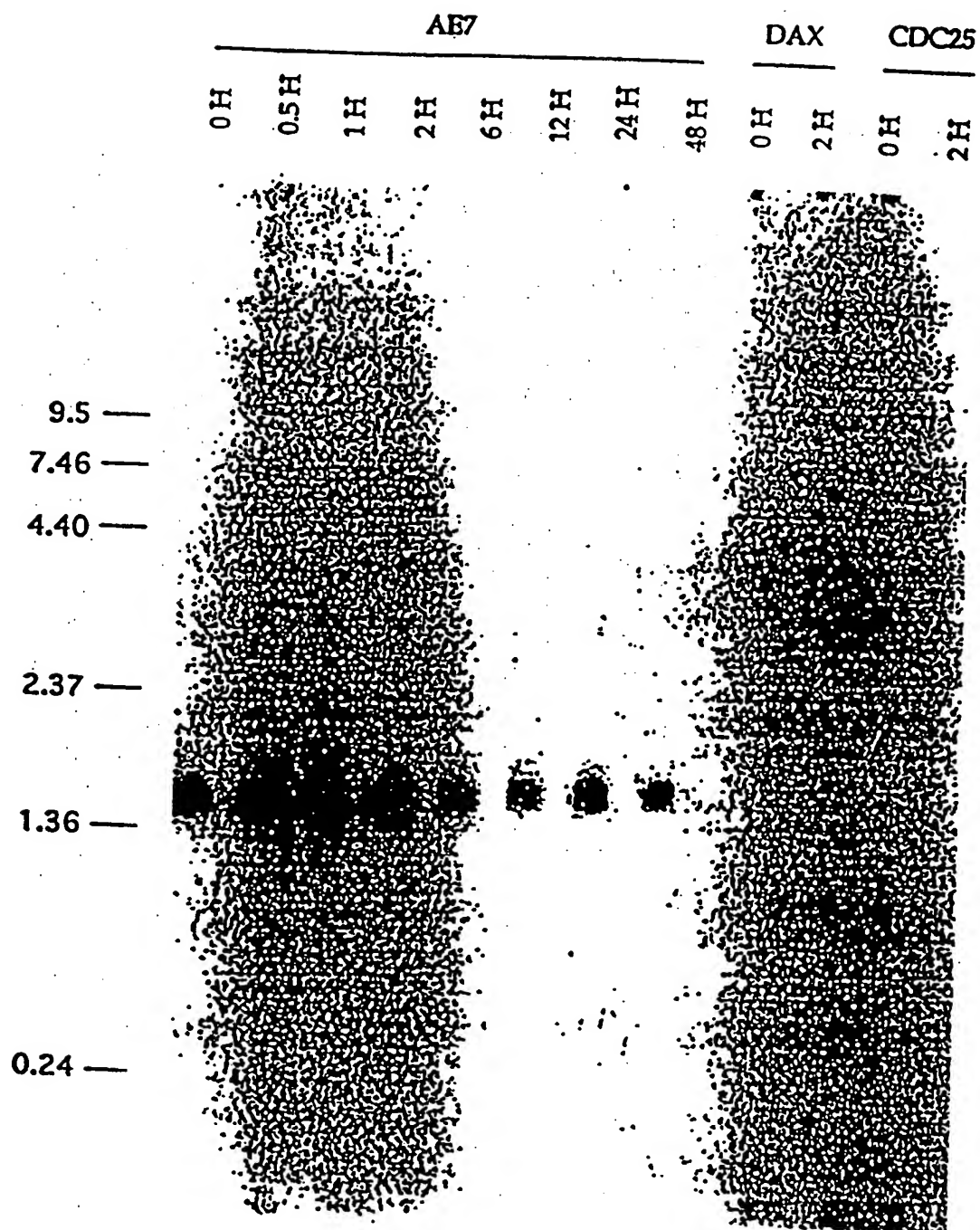


FIG. 20

31 / 47

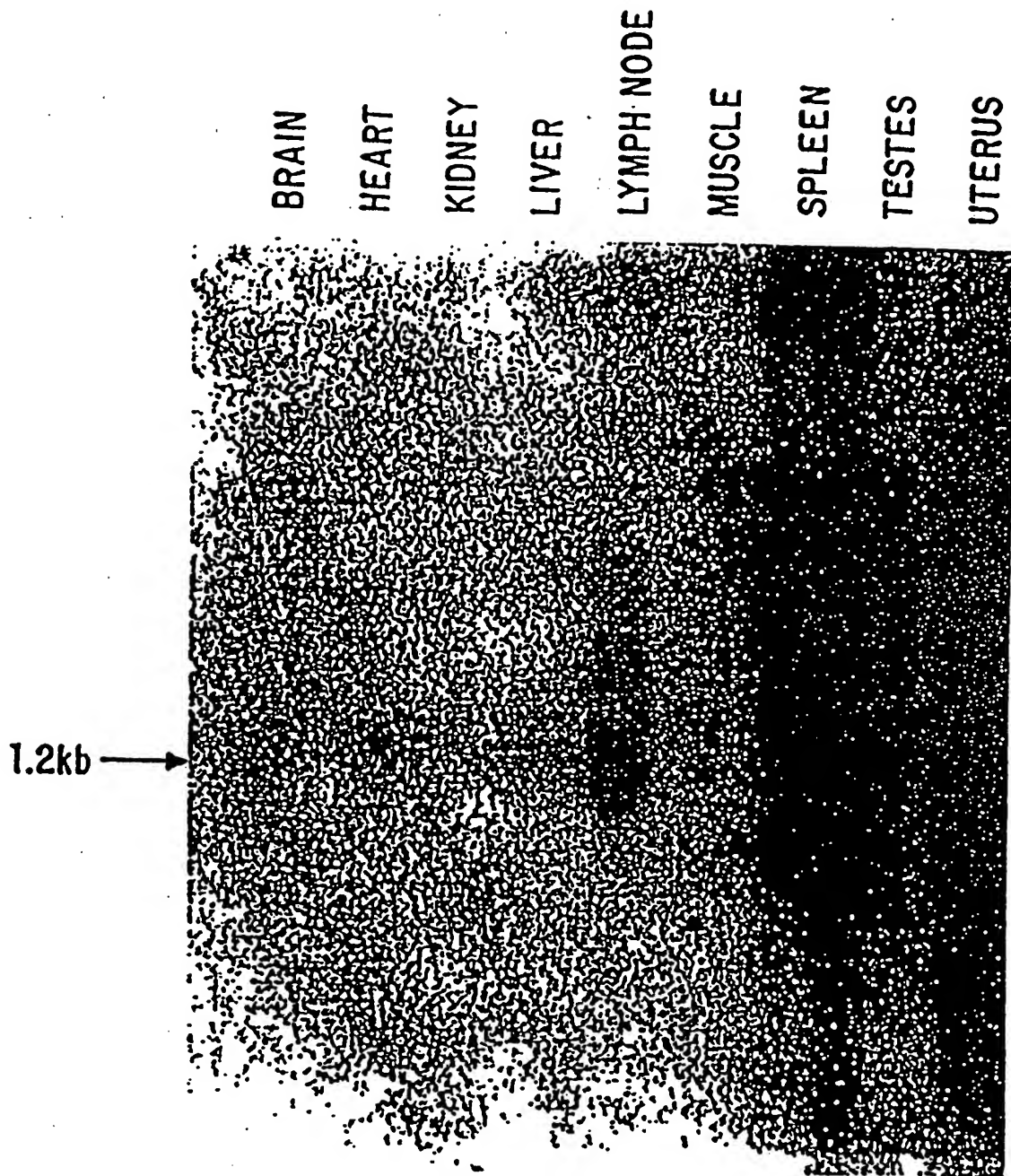


FIG. 21

```

      M T L T A H L S Y F L V L 13
C CGGGTCGACC CACGGGTCCG ATG ACA CTG ACT GCC CAC CTC TCC TAC TTT CTG GTC CTG 60

L L A G Q G L S D S L L T K D A G P R P 33
TTG TTA GCG GGC CAA GGC CTC AGT GAC TCC CTC CTC ACC AAG GAT GCA GGT CCC CGC CCA 120

L E L K E V F K L F Q I R F N R S Y W N 53
CTG GAG CTG AAG GAA GTC TTC AAG CTG TTC CAG ATC CGG TTC AAC CGG AGT TAC TGG AAC 180

P A E Y T R R L S I F A H N L A Q A Q R 73
CCA GCA GAG TAC ACT CGC CGT CTG AGC ATC TTT GCC CAC AAT CTG GCT CAG GCT CAA AGG 240

L Q Q E D L G T A E F G E T P F S D L T 93
CTA CAG CAA GAA GAC TTG GGT ACA GCT GAG TTT GGA GAG ACT CCA TTC AGT GAC CTC ACA 300

E E E F G Q L Y G Q E R S P E R T P N M 113
GAG GAG GAG TTT GGC CAG TTA TAC GGG CAG GAG AGG TCA CCA GAA AGG ACC CCC AAC ATG 360

T K K V E S N T W G E S V P R T C D W R 133
ACC AAA AAG GTA GAG TCT AAC ACG TGG GGG GAA TCT GTG CCC CGC ACC TGT GAC TGG CGT 420

K A K N I I S S V K N Q G S C K C W A 153
AAA GCA AAG AAC ATC ATC TCG TCG GTC AAG AAC CAG GGA AGC TGC AAA TGC TGC TGG GCC 480

M A A A D N I Q A L W R I K H Q Q F V D 173
ATG GCA GCT GCC GAC AAC ATC CAG GCT CTG TGG CGC ATC AAA CAC CAG TTT GTG GAC 540

```

FIG. 22A

V S V Q E L L D C E R C G N G C N G G F 193
 GTC TCT GTG CAG GAG CTG CTG GAC TGC GAA CGC TGT GGA AAT GGT TGC AAT GGT GGC TTC 600

 V W D A Y L T V L N N S G L A S E K D Y 213
 GTG TGG GAC GCA TAT CTA ACT GTC CTC AAC AAC AGT GGC CTG GCC AGT GAA AAG GAT TAT 660

 P F Q G D R K P H R C L A K K Y K K V A 233
 CCA TTC CAG GGG GAC AGA AAG CCT CAC AGA TGC CTA GCC AAG AAG TAC AAG AAG GTG GCC 720

 W I Q D F T M L S N N E Q A I A H Y L A 253
 TGG ATC CAG GAT TTC ACC ATG TTG TCC AAT AAT GAG CAG GCA ATT GCC CAC TAC CTG GCC 780

 V H G P I T V T I N M K L L Q H Y Q K G 273
 GTG CAT GGA CCT ATC ACC GTG ACC ATC AAC ATG AAA CTA CTC CAG CAT TAC CAG AAG GGT 840

 V I K A T P S S C D P R Q V D H S V L L 293
 GTC ATC AAG GCT ACA CCC AGC TCC TGT GAC CCT CGG CAA GTG GAC CAC TCT GTC TTG CTG 900

 V G F G K E K E G M Q T G T V L S H S R 313
 GTG GGC TTT GGC AAG GAG AAA GAG GGC ATG CAG ACA GGG ACA GTC TTG TCC CAT TCT CGA 960

 K R R H S S P Y W I L K N S W G A H W G 333
 AAA CGT CGC CAC TCC TCC CCA TAC TGG ATC CTG AAG AAC TCC TGG GGA GCT CAC TGG GGC 1020

 E K G Y F R L Y R G N N T C G V T K Y P 353
 GAG AAG GGT TAC TTC AGG CTG TAT CGG GGA AAC AAC ACC TGT GGA GTC ACC AAG TAT CCC 1080

FIG. 22B

34 / 47

F T A Q V D S P V K K A R T S C P P * 371
TTC ACA GCT CAA GTG GAC TCA CCA GTA AAG AAG GCA CGG ACC TCT TGT CCT CCC TGA AGG 1140
CAGCAGVCAC TCTTCTGCTT CTCCACATG GCCACTGCC CTTGTCAGCC CTGCCACAT CCTCTCTGTA 1210
TGGCTTCATA AACCAAGACT GCTCCGTGAA AAAAAAAAAAAAAAAA 1257

FIG. 22C

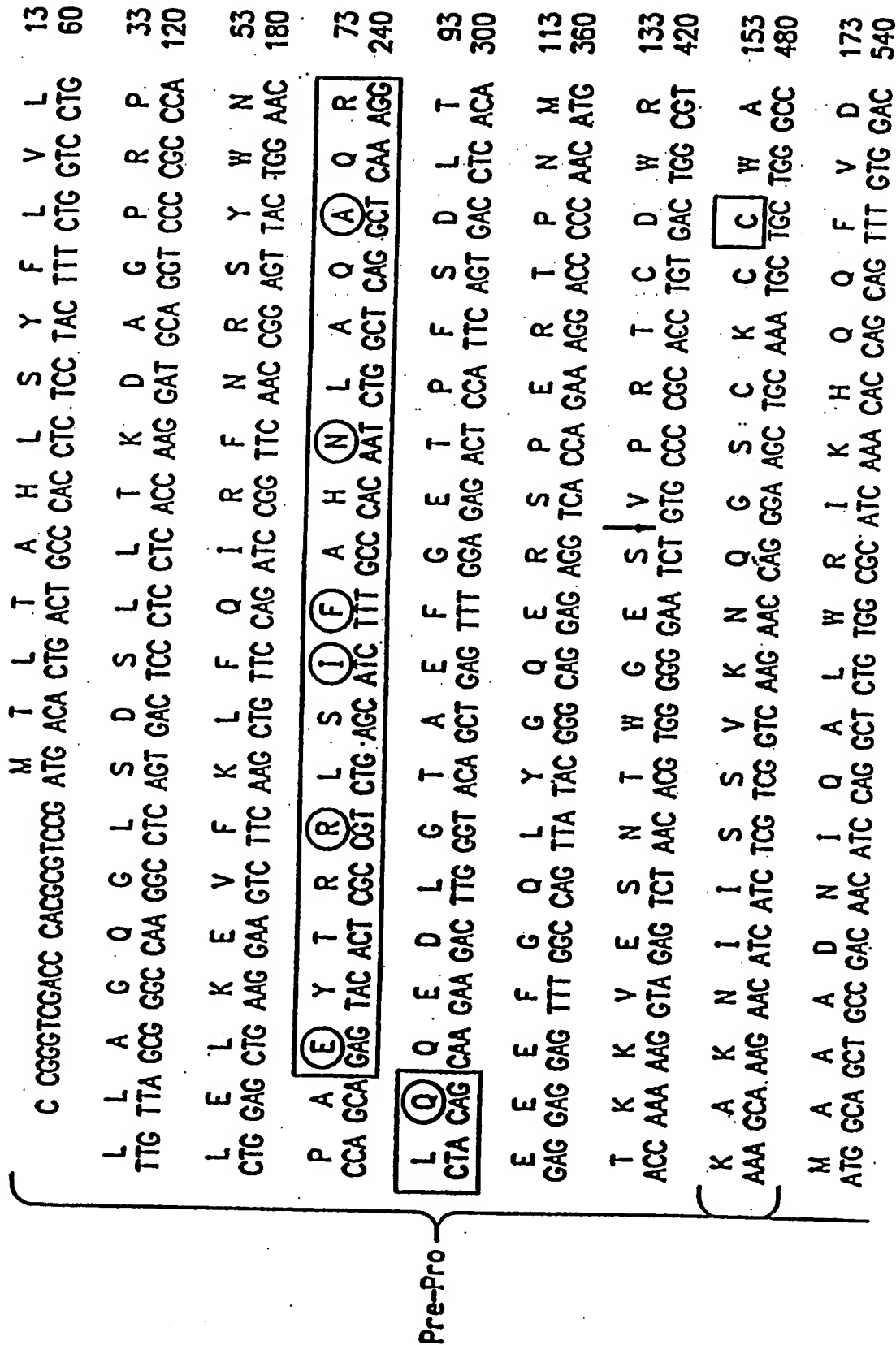


FIG. 23A

V S V Q E L L D C E R C G N G C N G G F 193
 GTC TCT GTG CAG GAG CTG CTG GAC TGC GAA CGC TGT GGA AAT GGT TGC AAT GGT GGC TTC 600
 V W D A Y L T V L N N S G L A S E K D Y 213
 GTG TGG GAC GCA TAT CTA ACT GTC CTC AAC AAC AGT GGC CTG GCC AGT GAA AAG GAT TAT 660
 P F Q G D R K P H R C L A K K Y K K V A 233
 CCA TTC CAG GGG GAC AGA AAG CCT CAC AGA TGC CTA GCC AAG AAG TAC AAG AAG GTG GCC 720
 W I Q D F T M L S N N E Q A I A H Y L A 253
 TGG ATC CAG GAT TTC ACC ATG TTG TCC AAT AAT GAG CAG GCA ATT GCC CAC TAC CTG GCC 780
 V H G P I T V T I N M K L L Q H Y Q K G 273
 GTG CAT GGA CCT ATC ACC GTG ACC ATC AAC ATG AAA CTA CTC CAG CAT TAC CAG AAG GGT 840
 V I K A T P S S C D P R Q V D S V L L 293
 GTC ATC AAG GCT ACA CCC AGC TCC TGT GAC CCT CCG CAA GTG GAC CAC TCT GTC TTG CTG 900
 V G F G K E K E G M Q T G T V L S H S R 313
 GTG GGC TTT GGC AAG GAG AAA GAG GGC ATG CAG ACA GCG ACA GTC TTG TCC CAT TCT CGA 960
 K R R H S S P Y W I L K N S W G A H W G 333
 AAA CGT CGC CAC TCC TCC CCA TAC TGG ATC CTG AAG AAC TCC TGG GGA GCT CAC TGG GGC 1020

MATURE

FIG.23B

37 / 47

E K G Y F R L Y R G N N T C G V T K Y P 353
GAG AAG GGT TAC TTC AGG CTG TAT CGG GGA AAC AAC ACC TGT GGA GTC ACC AAG TAT CCC 1080

F T A Q V D S P V K K A R T S C P P * 371
TTC ACA GCT CAA GTG GAC TCA CCA GTA AAG AAG GCA CGG ACC TCT TGT CCT CCC TGA AGG 1140

CAGCAGVCAC TCTTCTGCTT CTCCACATG GCCACTGCCC CTTGTCAGCC CTGCCACAT CCTCTCTGTA 1210

TGGCTTCATA AACCAAGACT GCTCCGTGAA AAAAAAAAAAAAAAA 1257

FIG.23C

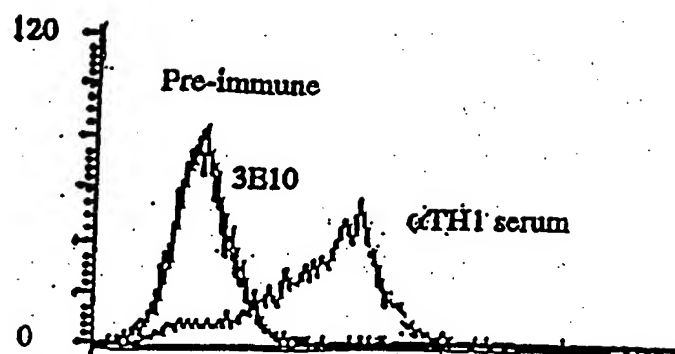
CGCTAACAGAGGTGTCTCTGACTTTTCTCTGCAAGCTOC																								M	F	S	H	L	P		6
																								ATG	TTT	TCA	CAT	CTT	CCC		18
F	D	C	V	L	L	L	L	L	L	L	T	R	S	S	E	V	E	Y		26											
TTT	GAC	TGT	GTC	CTG	CTG	CTG	CTG	CTA	CTA	CTT	ACA	AGG	TCC	TCA	GAA	GTG	GAA	TAC		78											
R	A	E	V	G	Q	N	A	Y	L	P	C	F	Y	T	P	A	A	P	G	46											
AGA	GCG	GAG	GTC	GGT	CAG	AAT	GCC	TAT	CTG	CCC	TGC	TTC	TAC	ACC	CCA	GCC	GCC	CCA	GGG	138											
N	L	V	P	V	C	W	G	K	G	A	C	P	V	F	E	C	G	N	V	66											
AAC	CTC	GTG	CCC	GTC	TGC	TGG	GGC	AAA	GGA	GCC	TGT	OCT	GTG	TTT	GAA	TGT	GGC	AAC	GTG	198											
V	L	R	T	D	E	R	D	V	N	Y	W	T	S	R	Y	W	L	N	G	86											
GTG	CTC	AGG	ACT	GAT	GAA	AGG	GAT	GTG	AAT	TAT	TGG	ACA	TCC	AGA	TAC	TGG	CTA	AAT	GGG	258											
D	F	R	K	G	D	V	S	L	T	I	E	N	V	T	L	A	D	S	G	106											
GAT	TTC	CGC	AAA	GGA	GAT	GTG	TCC	CTG	ACC	ATA	GAG	AAT	GTG	ACT	CTA	GCA	GAC	AGT	GGG	318											
I	Y	C	C	R	I	Q	I	P	G	I	M	N	D	E	K	F	N	L	K	126											
ATC	TAC	TGC	TGC	CGG	ATC	CAA	ATC	CCA	GGC	ATA	ATG	AAT	GAT	GAA	AAA	TTT	AAC	CTG	AAG	378											
L	V	I	K	P	A	K	V	T	P	A	P	T	L	Q	R	D	F	T	A	146											
TTG	GTC	ATC	AAA	CCA	GCC	AAG	GTC	ACC	OCT	GCA	CCG	ACT	CTG	CAG	AGA	GAC	TTC	ACT	GCA	438											
A	F	P	R	M	L	T	T	R	G	H	G	P	A	E	T	Q	T	L	G	166											
GCC	TTT	CCA	AGG	ATG	CTT	ACC	ACC	AGG	GGA	CAT	GGC	CCA	GCA	GAG	ACA	CAG	ACA	CTG	GGG	498											
S	L	P	D	I	N	L	T	Q	I	S	T	L	A	N	E	L	R	D	S	186											
AGC	CTC	OCT	GAT	ATA	AAT	CTA	ACA	CAA	ATA	TCC	ACA	TTG	GCC	AAT	GAG	TTA	CGG	GAC	TCT	558											
R	L	A	N	D	L	R	D	S	G	A	T	I	R	I	G	I	Y	I	G	206											
AGA	TTG	GCC	AAT	GAC	TTA	CGG	GAC	TCT	GGA	GCA	ACC	ATC	AGA	ATA	GCC	ATC	TAC	ATC	GGA	618											
A	G	I	C	A	G	L	A	L	A	L	I	F	G	A	L	I	F	K	W	226											
GCA	GGG	ATC	TGT	GCT	GGG	CTG	GCT	CTG	GCT	CTT	ATC	TTC	GGC	GCT	TTA	ATT	TTC	AAA	TGG	678											
Y	S	H	S	K	E	K	I	Q	N	L	S	L	I	S	L	A	N	L	P	246											
TAT	TCT	CAT	AGC	AAA	GAG	AAG	ATA	CAG	AAT	TTA	AGC	CTC	ATC	TCT	TTG	GCC	AAC	CTC	OCT	738											
P	S	G	L	A	N	A	V	A	E	G	I	R	S	E	E	N	I	Y	T	266											
CCC	TCA	GGA	TTG	GCA	AAT	GCA	GTA	GCA	GAG	GGA	ATT	CCC	TCA	GAA	GAA	AAC	ATC	TAT	ACC	798											
I	E	E	N	V	Y	E	V	E	E	P	N	E	Y	Y	C	Y	V	S	S	286											
ATT	GAA	GAG	AAC	GTA	TAT	GAA	GTG	GAG	GAG	CCC	AAT	GAG	TAT	TAT	TGC	TAT	GTC	AGC	AGC	858											
R	Q	Q	P	S	Q	P	L	G	C	R	F	A	M	P						301											
AGG	CAG	CAA	CCC	TCA	CAA	OCT	TTG	GGT	TGT	CGC	TTT	GCA	ATG	CCA	TAGAT	CCAA	CCCTT	ATT		903											
TTTGAAGCTTGGTGTGTTTTGTCTTTTTTCAGAACTATGAGCTGTGTCAOCTGACTGGTTTTGGAGGTTCTGTCTCACTGCTA																															
TGGAGCAGAGTTTTCCCATTTTTTCAGAACTAATGACTCAGATGGGAATTGAACTGGGAOCTGCACTGAACCTTAACAGG																															
CATGTCTATTGCTCTGTATTTAAGCCAAACAGAGTTAACCACCCAGAGACTGTTAATCATGGATGTTAGAGCTCAAAAG																															
GGCTTTTATATACACTAGGAATTTCTGAAGTGGGGTCTCTGGAGCTOCAGGAATTOGGG CACATCATATGTCCATGA																															
AACTTCAGATAAACTAGGAAAACTGGGTCTGAGGTGAAAGCATAACTTTTTTGGCACAGAAAGTCTAAAGGGGGCCAC																															
TGATTTTCAAAGAGATCTGTGATOOCTTTTTGTGTTTTGTGTTTTGAGATGGAGTCTTCTCTGTGTGOCAGGGCTGGAGT																															
GCAATGCCACAATCTGGGCTCACTOCAGCTOOGGCTOCTGGGTTCAAGGATTTCTOCTGGOCTCAGGCTOCTGAGTGGC																															
TGGGATTACAGCCATOCACCCACATCCOCCAGCTAAATTGTTGTATTTTTAGTAGAGACAGGGTTTTCAOCCATGTTGGCCA																															

GTGTGGTCTCAAACTOCTGAOCTCATGATTTGCTGCTOCTGGGCTOCCAAAGCACTGGGATTACAGGGGTGAGOCAOCA
CATOCAGOCAGTGATCCTTAAAGATTAAGAGATGACTGGACTAGGTCTACCTTGATCTTGAAGATTCCCTTGGAAATGT
TGAGATTAGGCTTATTTGAGCACTAOCCTGOCCTACTGTCTAGTGOCAGTGCCATAGOCCTTCTTTTGTCTOCTTATGAA
GACTGOCCTGCAGGGCTGAGATGTGGCAGGAGCTOCCAGGGAAAAAGGAAGTGCAATTGATTGGTGTGTATTGGOCAAG
TTTGTCTGTGTGTGTGCTTGAAGAAAAATATCTCTGAOCTTCTGTATTGGTGGACAACTGAAGCTATATTTTC
ACAGAAGAAGAAGCAGTGACGGGGACACAAATTCTGTTGCTGCTGGAAAGAAGGCAAGGCTTCAGCAATCTATATT
AOCAGOGCTGGATCCTTTGACAGAGAGTGGTCCCTAACTTAAATTTCAGAGOGGTATAGGCTTGATCTGTCTTGTCTTA
TTGTTGOCCTGOGCTAGCACAAATCTGACACACAATTGGAACCTTACTAAAAATTTTTTTTACTGTTAAAAAAA
AAAAAAA

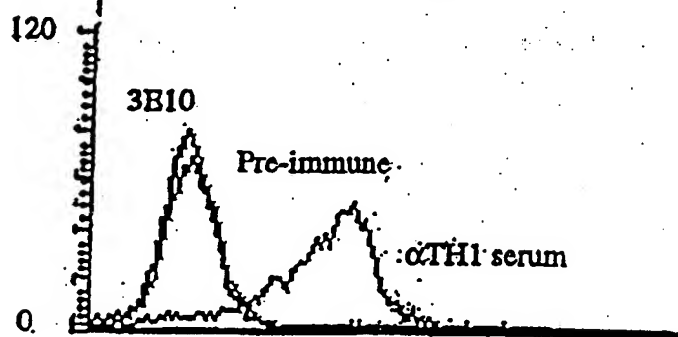
FIG. 24 (cont'd.)

40 / 47

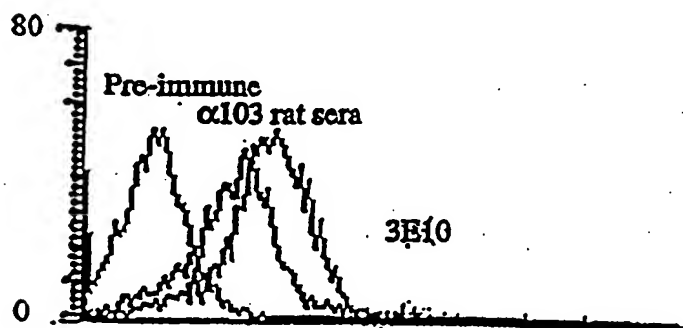
AE7



Dorris



D10.G4



DAX

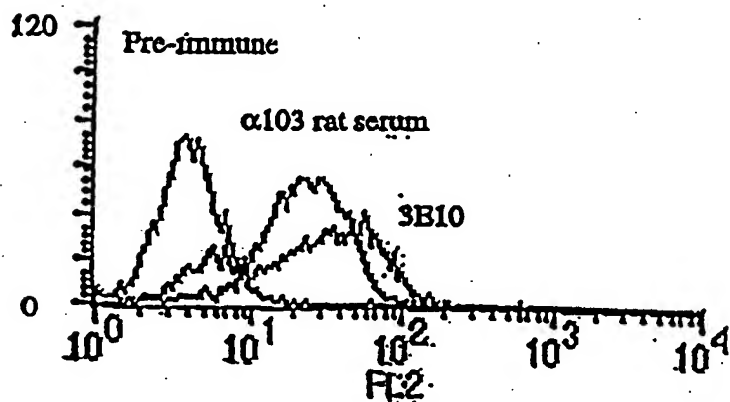


FIGURE 25

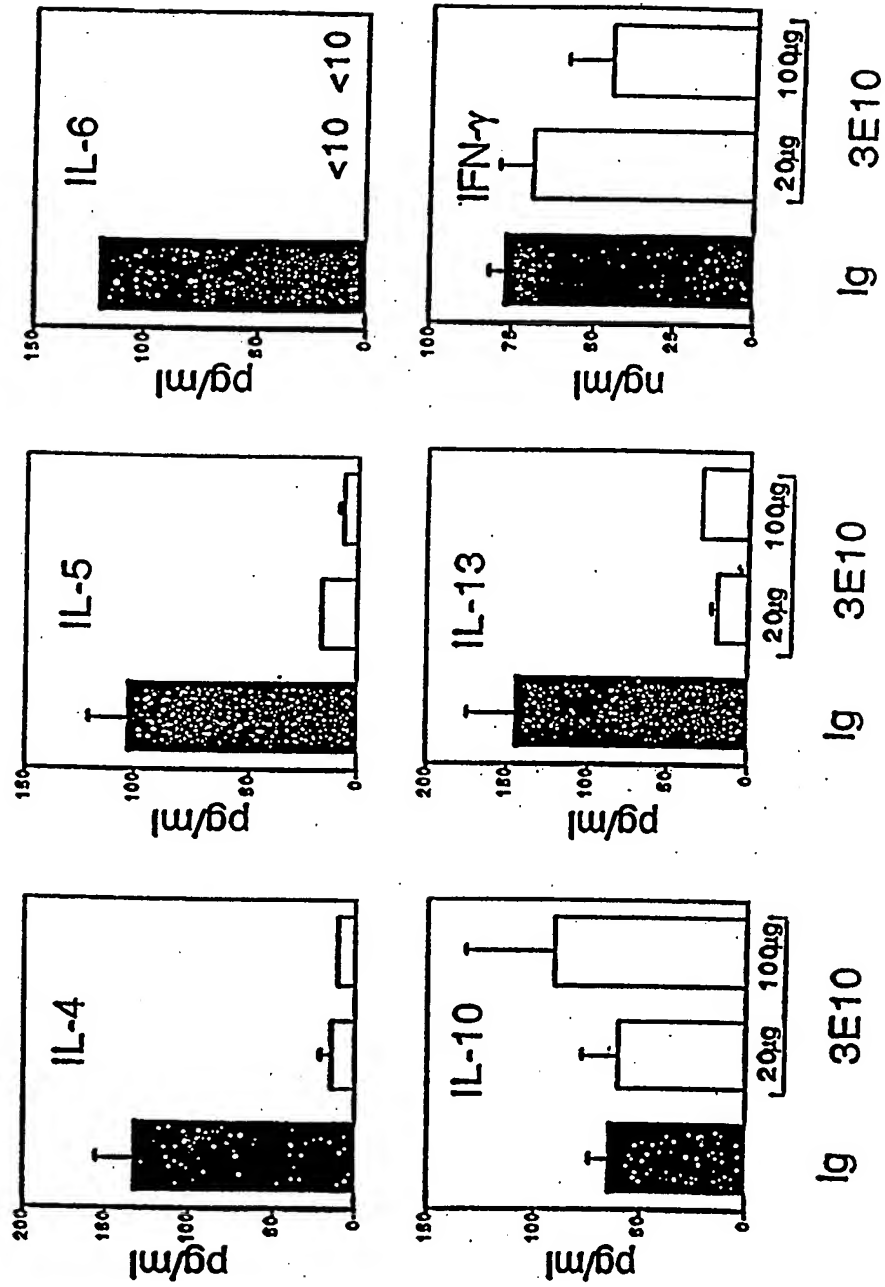


FIGURE 26

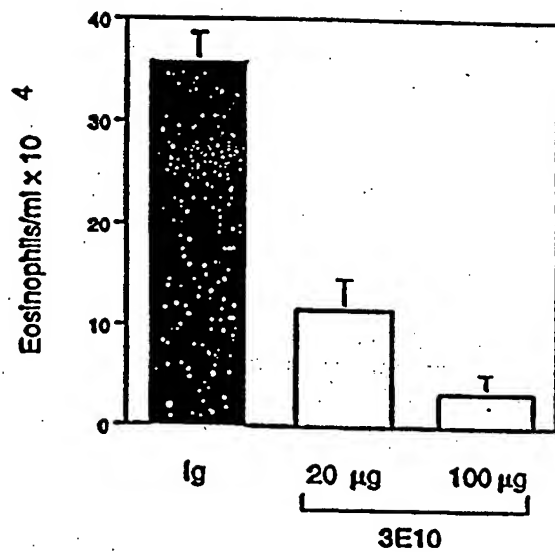


FIGURE 27A

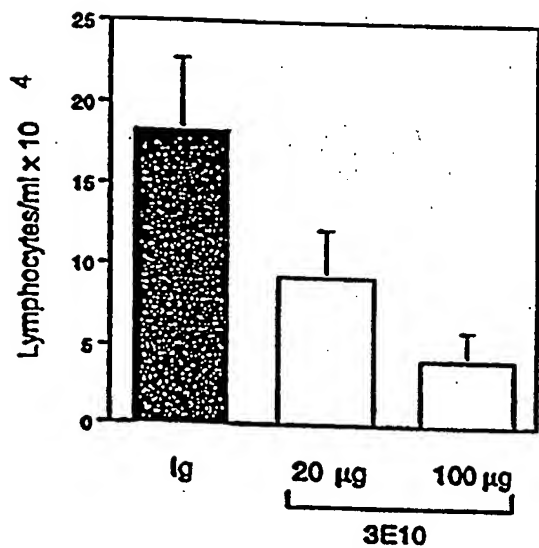


FIGURE 27B

43 / 47

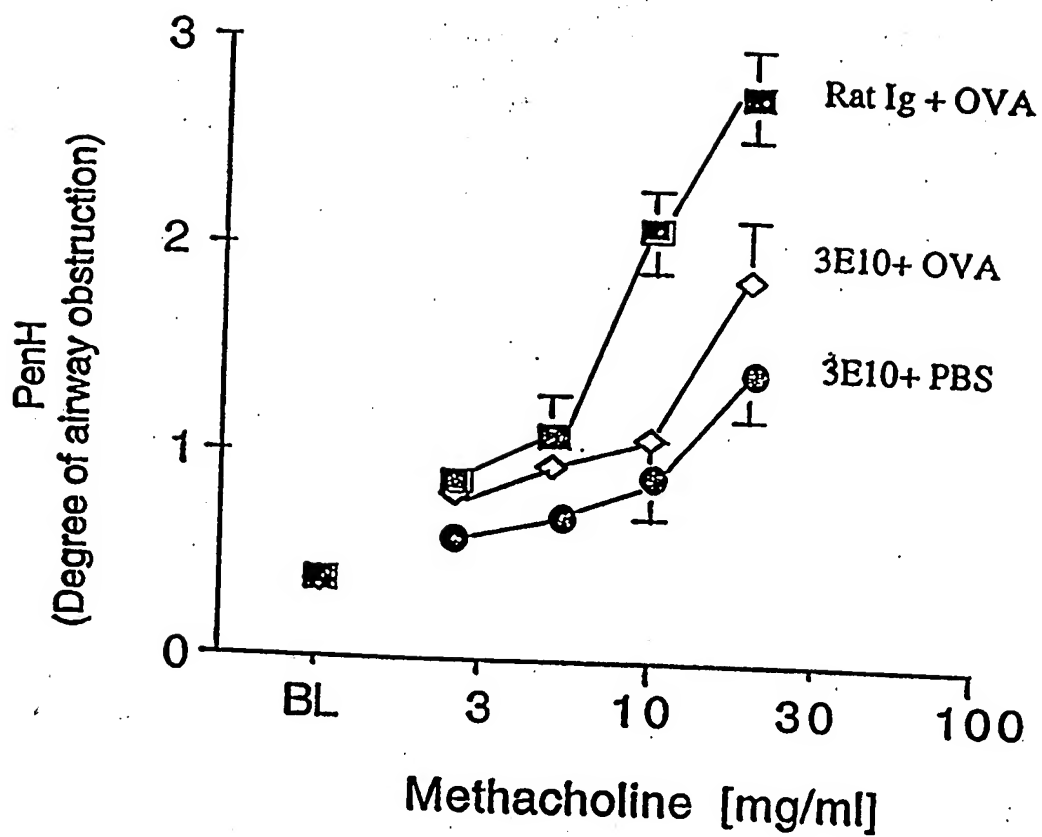


FIGURE 2B

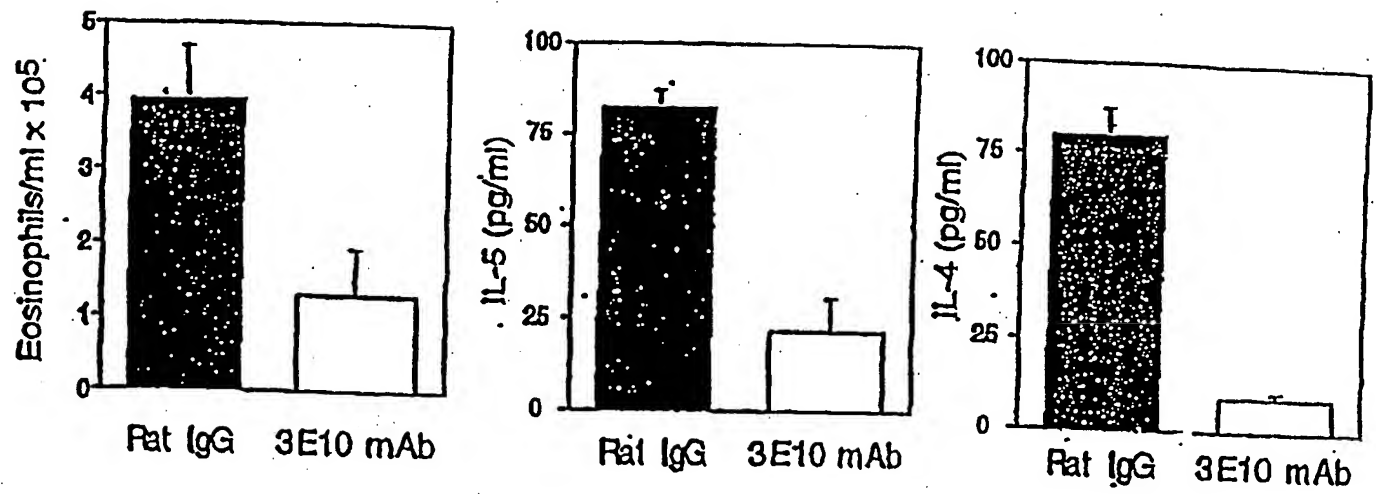


FIGURE 29A

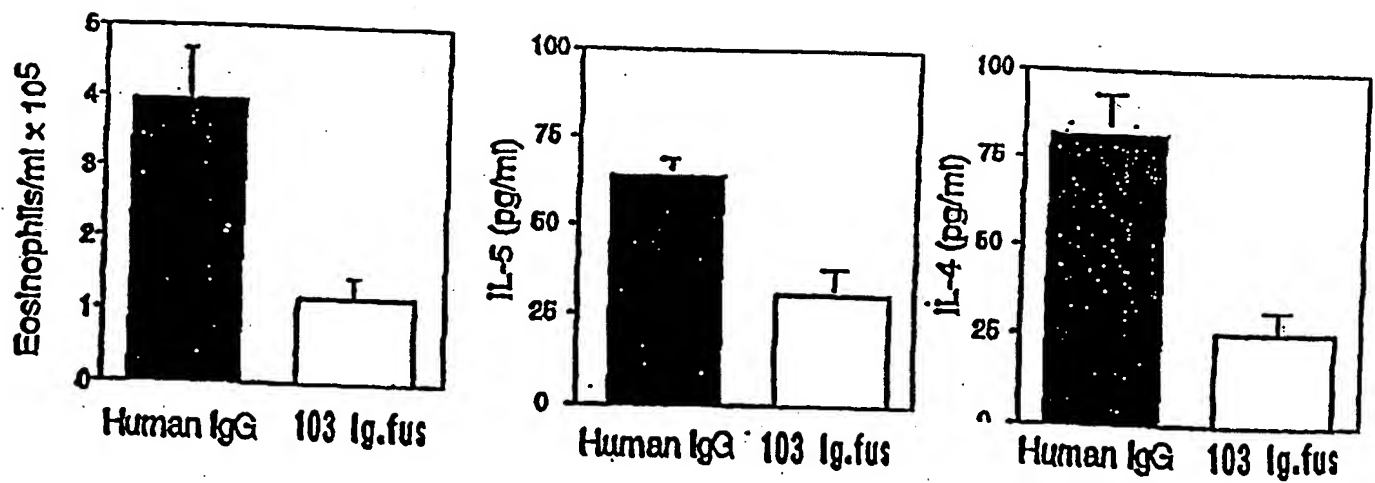


FIGURE 29B

45 / 47

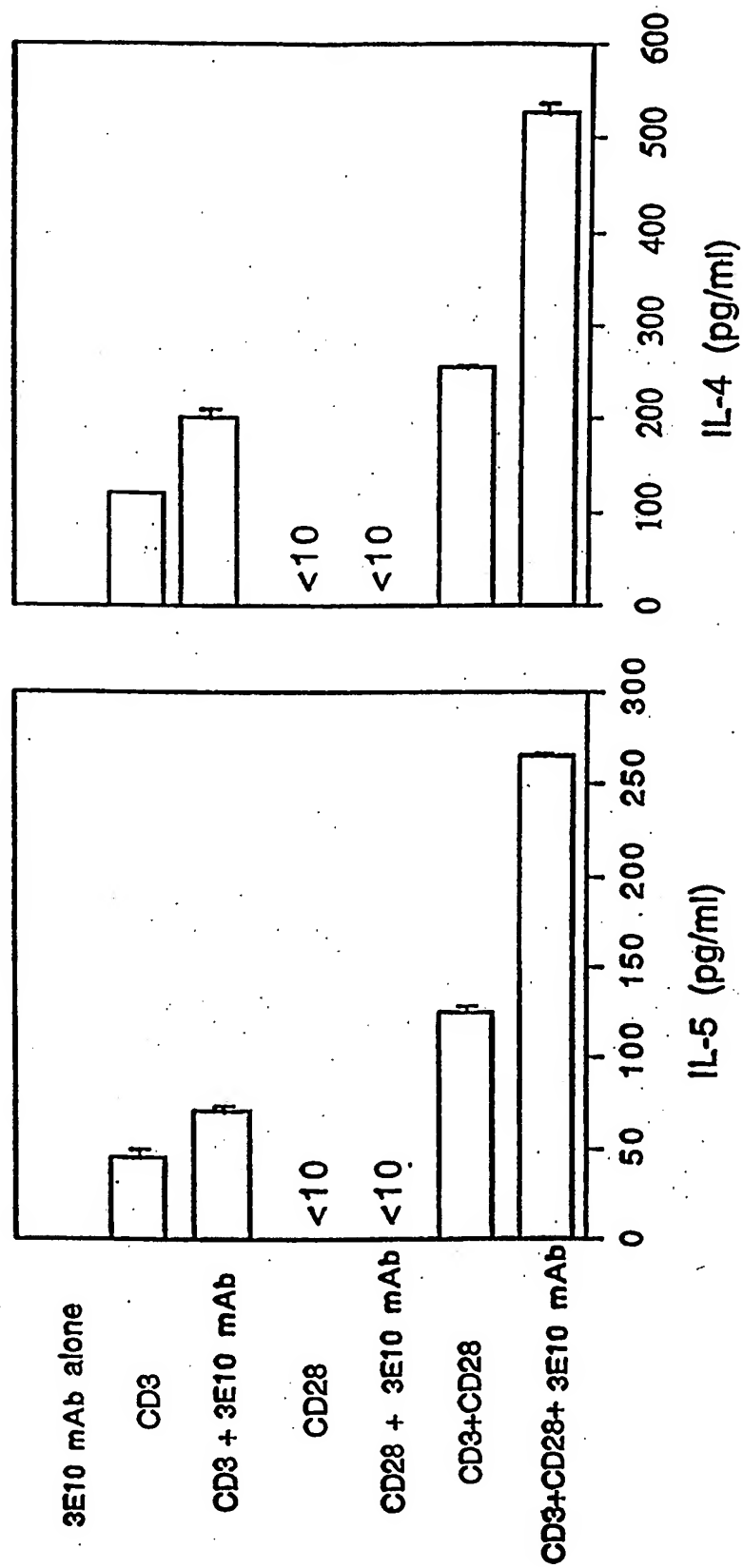
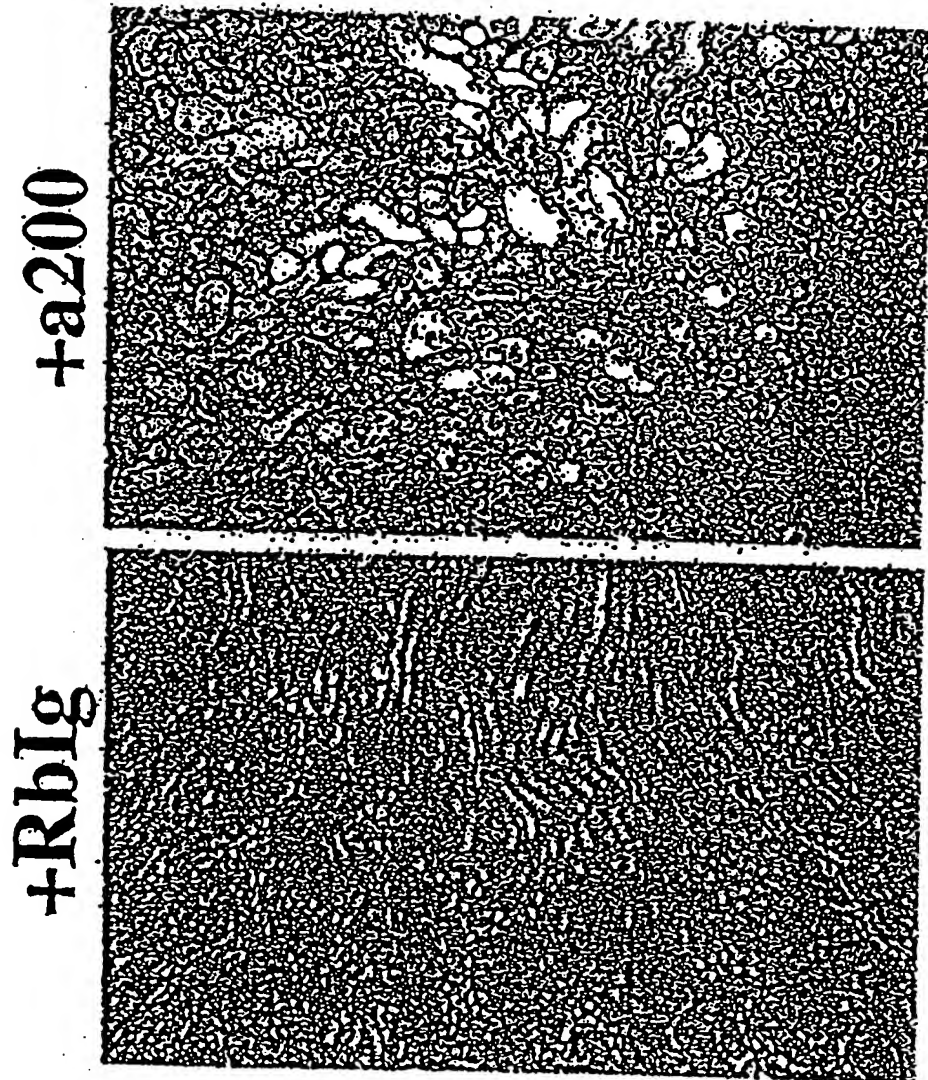


FIG. 30

Renal histology at 72hrs post reperfusion

**FIG. 31**

Blockage of gene 200 during renal ischemia/reperfusion injury

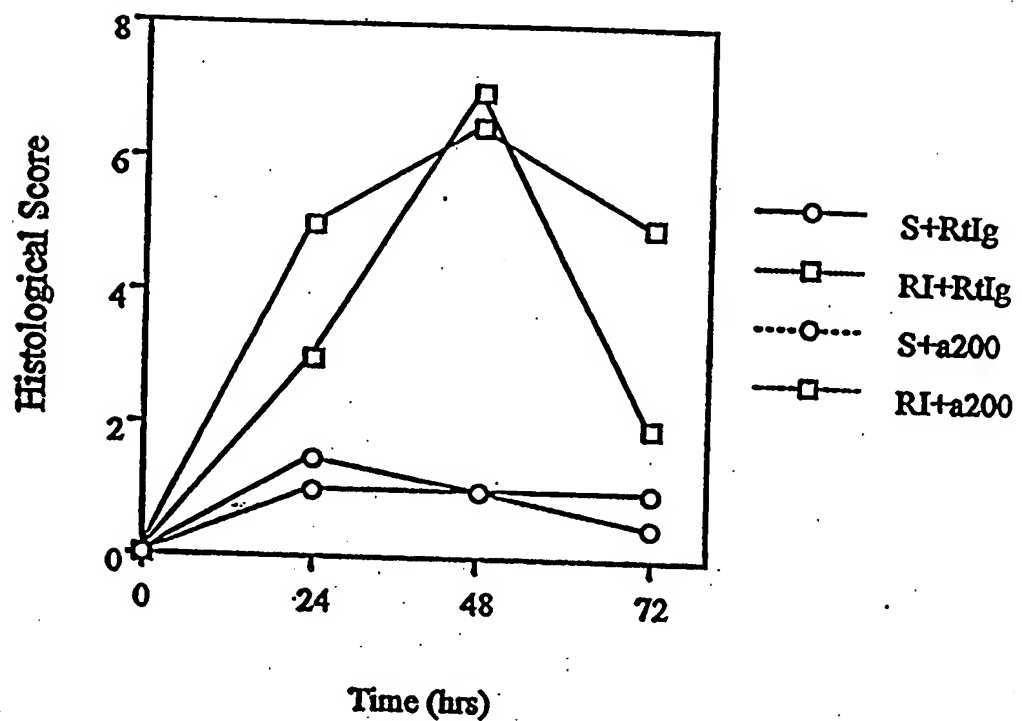


FIG. 32

SEQUENCE LISTING

<110> Millennium Pharmaceuticals, Inc.

<120> COMPOSITIONS AND METHODS FOR THE TREATMENT AND
DIAGNOSIS OF IMMUNE DISORDERS

<130> 7853-125-228

<140>

<141>

<150> 09/324,986

<151> 1999-06-02

<160> 49

<170> PatentIn Ver. 2.0

<210> 1

<211> 357

<212> DNA

<213> Mus musculus

<400> 1

ctgggtgaggg ggatctacaa cttgttcggt taaagaaaa agcaacagcc aacagaaatg 60
tggttatacct tcacctacct aaaaaggag atgatgtgaa accaggaacc agatgccgag 120
tagcaggatg ggggagattt ggcaataagt cagctccctc tgaaactctg agagaagtca 180
acatcactgt catagacaga aaaatctgca atgatgaaaa acactataat tttcatcctg 240
taattggtct aaacatgatt tgggcagggg acctccccgg cggaaaggac tcctgcaatg 300
gggattctgg cagccctctc ctatgtgatt ggtatttggg aagcatcacc tcctttt 357

<210> 2

<211> 255

<212> DNA

<213> Mus musculus

<400> 2

ttagcgccat tgccatagag agacctcagc catcaatcac tagcacatga ttgacagaca 60
gagaatggga ctttgggctt tggcaattct gacacttccc atgtatttga cagttacgga 120
gggcagtaaa tcgtcctggg gtctggaaaa tgaggcttta attgtgagat gcccccaaag 180
aggacgctcg acttatcctg tggaatggta ttactcagat acaaataaaa gtattcctac 240
ccaaaaaaaa aaaaaa 255

<210> 3

<211> 2055

<212> DNA

<213> Mus musculus

<400> 3

ccgggtcgac ccacgcgtcc gagcctcctc agtcaagaga agcatccctc cagaaacagg 60
gaaacatgac acttttgaaa gaatgccaaa cggcgtgaaa ataaaaacag agcattccca 120
tttgaccga ccaatctcca atctcctgta agattcaaaa gggcaagcaa gaggcggtga 180
ccgttcacga aagctaaaat cccatgctat tgaacatgaa gacttctgat gcttaaactct 240
cattaactgc tttaagtcac tcccaggagc ttggatccca acttctagca gtaatagtct 300
gtgtaaaaaa aaaaaaaaaa tcagtctaca accactctct aaatgcatgg atgaactcat 360
cagaacatca aaaccaagg aaaccctaag agagaagaat tctaataaaa agaattttac 420
attgaaaact tacaaggcaa ggtccctttc cctgctgaca gcctaagaag tgatgtaact 480
gccactgtga agaccatggc gatgaacagc atgtgcattg aagagcagcg ccacctcgaa 540
cactatttgt tcccggtggt ctacataatt gtgtttatag tcagcgtccc agccaacatc 600
ggatctttat gcgtatcctt tctgcaagcg aagaaggaaa atgagctagg gatttacctc 660
ttcagtctgt ccctgtcaga cctgctgtat gcgctgactc tgcccctctg gatcaattac 720
acttggaata aagacaactg gactttctct cccacctgtg gcaaaggaag cgttttcttc 780
acctacatga acttttacag cagcacggcg ttcctcactt gcattgccct ggaccgctat 840
ttagcagtcg tctaccctct gaagttttcc ttcctaagaa cgagaagatt cgcgtttatt 900
accagcctct ccatctggat attagagtc tcttttaact ctatgcttct gtggaaagat 960
gaaacgagtg ttgaatattg tgactcggac aaatctaatt tcaactctctg ctatgacaaa 1020
taccctctgg agaaatggca gataaacctc aacctgtttc ggacgtgcat gggctacgca 1080
atacccttga tcaccatcat gatctgcaac cataaagtct accgagctgt gcggcacaac 1140
caagccacgg aaaacagcga gaagagaagg atcataaagt tgcttgctag catcacgttg 1200
actttcgtcc tatgctttac ccccttcac gtgatggtgc tcatccgctg cgtttttagag 1260
cgcgacatga acgtcaatga caagtctgga tggcagacgt ttacggtgta cagagtcaca 1320
gtagccctga cgagtctaaa ctgtgttgcc gatcccatc tgtactgctt tgtgactgag 1380
acgggggagag ctgatatgtg gaacatatta aaattgtgta ctaggaaaca caatagacac 1440
caagggaaaa aaaggacat actttctgtg tccacaagag atgctgtaga attagagatt 1500
atagactaag aggtggaggc aggttaagtt acatgggtatt atttaatgaa acttacattt 1560
tggaagaa atctggcata gtagaaccca gtggaaatag tttgaaggta cattgtatga 1620

ctcctatggt ggctttatta agtaaggat agaaatgtat tatcttgat gtattcta 1680
gactaggcat cattgtttta gtaccaattc tctttgctc tatgttataa cccctaagaa 1740
gcacgcggga ctgttcgtct ttaaatacgt ggccattcta tctgactact atgacttttt 1800
gttggtgttc tgctttgggt tttcagtcg cctgcatcag tcttctctc tgtatacgtc 1860
tgtcttcaac aaatgtaagg actaaatacc cctcccgatc acatccatta tcaaggattt 1920
gaagccactc catgtactgg gttataaaaag aaatgttctc atgaactttc atgaagttaa 1980
catacctttg gggatctagt caccgagtca cataaagtaa aagtaaattg aaaaaaaaaa 2040
aaaaaaaaaa agggc 2055

<210> 4

<211> 460

<212> DNA

<213> Mus musculus

<220>

<221> modified_base

<222> all "n" positions

<223> n=a, c, g, or t

<400> 4

cgccagtgtg ctggaattcg gcttagagca tttctttcaa accacagggt aacacacact 60
tactaaaaag caatgctgtt agaggagaag ggcttgggag actcggccat ttgaaacana 120
agcaaggcac tctccaggnn cagcaagtgg attcccattt cctgctgagg gcgggttcac 180
actgagactg cactccagtc agcgggagga atcacctgca ttaatgcttg tcctctgcag 240
agctagtgtg ccttccactc tgggtacact tgggtgtcaa catttcaaaa tgatgaccta 300
agaggctctc atagttgggtg ataactatgg naggacagaa gaacactggc tgtattgtct 360
tttcttttca gcactagtgt cttggccctt aactaaaacg gggtccatca tcctccaaac 420
caggaagata gattgttaga caggtccttt cccctcaact 460

<210> 5

<211> 414

<212> DNA

<213> Mus musculus

<220>

<221> modified_base

<222> all "n" positions

<223> n=a, c, g, or t

<400> 5

tttttttttt tngggagagg ctagcactga aattacagtt tcagtggaaat ttagagaagt 60

aataactgca aaaatttatt tacacacaca cacacacaca cagggcattt tacctgtgta 120
 agtgcagttt aatcancccc attaccttat gaccttggtt ggcaatgtct ctaaagcttt 180
 aaaattaaaa taaaattaaa aagatgggtt tccatctcat aaaatcccct ttgggaatgg 240
 aagacttcct ctttggggtn ttttttagag ggaacaggag gtaactgtta attatttata 300
 cattctaata aaccatgaat gcaccacata aaatactgta ctcggggagc aaacactgtn 360
 tggggggggtt ctctcttacc agaaggaaca gggggctttt caatggctgt gggc 414

<210> 6
 <211> 240
 <212> DNA
 <213> Mus musculus

<220>
 <221> modified_base
 <222> all "n" positions
 <223> n=a, c, g, or t

<400> 6
 ttttngggac agggtttcnc tgtgtatctc tggtgtcct ggaactnact ctgtagacca 60
 gggtggcctc ganctcagaa atctacctgc ctctccctcc anagtgtgg gattaanggt 120
 gtagccacc aatncccggc cttaatatat tnntaaacaa cttcatttga atganatatt 180
 gacactaccc ttggaataag agtncccgga atgangtaca ggnttcangg aatcatttaa 240

<210> 7
 <211> 217
 <212> DNA
 <213> Mus musculus

<400> 7
 cttagcaggt ggagttgcag caggaagcct ggtagccaca ctccaatcag caggggtcct 60
 tggactctcc acatcaacaa atgccatcct aggggtgtgt ggggcactgt tggagccttg 120
 ctctgagctt aggagatgac acttctatca gctcaactca aagcctgtac agactacgca 180
 ggagatgaag ttccaaaagg caccttcaga accctca 217

<210> 8
 <211> 2710
 <212> DNA
 <213> Mus musculus

<220>
 <221> modified_base
 <222> all "n" positions
 <223> n=a, c, g, or t

<400> 8

ngtcgaccca cgcgtccgga tttccctcc caagtactca tgttttcagg tcttaccctc 60
aactgtgtcc tgctgctgct gcaactacta cttgcaaggt cattggaaga tggttataag 120
gttgagggttg gtaaaaatgc ctatctgccc tgcagttaca ctctacctac atctgggaca 180
cttgtgccta tgtgctgggg caagggattc tgtccttggc cacagtgtac caatgagttg 240
ctcagaactg atgaaagaaa tgtgacatat cagaaatcca gcagatacca gctaaagggc 300
gatctcaaca aaggagatgt gtctctgac ataaagaatg tgactctgga tgaccatggg 360
acctactgct gcaggatata gttccctggc cttatgaatg ataaaaaatt agaactgaaa 420
ttagacatca aagcagccaa ggtcactcca gctcagactg cccatgggga ctctactaca 480
gcttctccaa gaacctaac cacggagaga aatgggtcag agacacagac actggtgacc 540
ctccataata acaatggaac aaaaatttcc acatgggctg atgaaattaa ggactctgga 600
gaaacgatca gaactgctat ccacattgga gtgggagtct ctgctgggtt gacctggca 660
cttatcattg gtgtcttaat ccttaaatgg tattcctgta agaaaaagaa gttatcgagt 720
ttgagcctta ttactctggc caacttgctt ccaggagggt tggcaaatgc aggagcagtc 780
aggattcgct ctgaggaaaa tatctacacc atcgaggaga acgtatatga agtggagaat 840
tcaaatgagt actactgcta cgtcaacagc cagcagccat cctgaccgcc tctggactgc 900
cacttttaaa ggctcgctt catttctgac tttgggtattt cctttktgg aaaactatgt 960
gatatgtcac ttggcaacct cattggaggt tctgaccaca gccactgaga aaagagtcc 1020
agttttctgg ggataattaa ctcaacagg gattcgactg taactcatgc tacattgaaa 1080
tgctccattt tatccctgag tttcagggat cggatctccc actccagaga cttcaatcat 1140
gcgtgttgaa gctcactcgt gctttcatac attaggaatg gttagtgtga tgtctttgag 1200
acatagaggt ttgtggtata tccgcaaagc tcctgaacag gtagggggaa taaagggcta 1260
agataggaag gtgcggtctt tgttgatgtt ggaaaatctt aaagaagttg gtagcttttc 1320
tagagatttc tgacctgaa agattaagaa aaagccaggt ggcataatgt taacacgata 1380
taacttgga accttaggca ggagggtgat aagttcaagg tcagccaggg ctatgctggc 1440
aagactgtct camcatccaa agacgaaaat aaacatagag acagcaggag gctggagatg 1500
aggctcggac agtgaggtgc attgtgtaca agcagcagga atctatatatt gatcgtagac 1560
cccacatgaa aaagctaggc ctggtagagc atgctttag actcaagaga tggagaggta 1620
aaggcacaac agatccccgg ggcttgctg cagtcagctt agcctaggtg ctgagttcca 1680
agtcacaag agtccctgtc tcamagtaag atggrrctgag tatctggcgc atgtccatgg 1740

```

gggttgctcct ctctctcag aagagacatg cacatgwccc tgcacacaca cacacacaca 1800
cacacacaca cacacacaca cacacacaca tgawatgaag gttctctctg tgctgtctac 1860
ctctctataa catgtatctc tacaggactc tcctctgcct ctgttaagac atgagtggga 1920
gcatggcaga gcagtccagt aatttatcc agcactcaga aggctggagc agaagcgtgg 1980
agagttcagg agcactgtgc ccaacactgc cagactcttc ttacacaaga aaaagggttac 2040
ccgcaagcag cctgctgtct gtaaaaggaa accctgcgaa aggcaaactt tgactgttgt 2100
gtgctcaagg ggaactgact cagacaactt ctccattcct ggaggaaact ggagctgttt 2160
ctgacagaag aacaaccggt gactgggaca tacgaaggca gagctcttgc agcaatctat 2220
atagtcagca aaatattctt tgggaggaca gtcgtcacca aattgatttc caagccggtg 2280
gacctcagtt tcatctggct tacagctgcc tgcccagtgc ccttgatctg tgctggctcc 2340
catctataac agaatcaaat taaatagacc ccgagtgaat atattaagtg agcagaaagg 2400
tagctttggt caaagatttt tttgcattgg ggagcaactg tgtacatcag aggacatctg 2460
ttagtgagga caccaaaacc tgtggtaccg ttttttcatg tatgaatttt gttgtttagg 2520
ttgcttctag ctagctgtgg aggtcctggc tttcttaggt gggtatggaa gggagaccat 2580
ctaacaaaat ccattagaga taacagctct catgcagaag ggaaaactaa tctcaaattg 2640
tttaaagtaa taaaactgta ctggcaaagt actttgagca taaaaaaaaa aaaaaaaaaa 2700
gggcggccgc 2710

```

<210> 9
 <211> 337
 <212> PRT
 <213> Mus musculus

<400> 9
 Met Ala Met Asn Ser Met Cys Ile Glu Glu Gln Arg His Leu Glu His
 1 5 10 15
 Tyr Leu Phe Pro Val Val Tyr Ile Ile Val Phe Ile Val Ser Val Pro
 20 25 30
 Ala Asn Ile Gly Ser Leu Cys Val Ser Phe Leu Gln Ala Lys Lys Glu
 35 40 45
 Asn Glu Leu Gly Ile Tyr Leu Phe Ser Leu Ser Leu Ser Asp Leu Leu
 50 55 60
 Tyr Ala Leu Thr Leu Pro Leu Trp Ile Asn Tyr Thr Trp Asn Lys Asp
 65 70 75 80
 Asn Trp Thr Phe Ser Pro Thr Leu Cys Lys Gly Ser Val Phe Phe Thr
 85 90 95

Tyr Met Asn Phe Tyr Ser Ser Thr Ala Phe Leu Thr Cys Ile Ala Leu
 100 105 110
 Asp Arg Tyr Leu Ala Val Val Tyr Pro Leu Lys Phe Ser Phe Leu Arg
 115 120 125
 Thr Arg Arg Phe Ala Phe Ile Thr Ser Leu Ser Ile Trp Ile Leu Glu
 130 135 140
 Ser Phe Phe Asn Ser Met Leu Leu Trp Lys Asp Glu Thr Ser Val Glu
 145 150 155 160
 Tyr Cys Asp Ser Asp Lys Ser Asn Phe Thr Leu Cys Tyr Asp Lys Tyr
 165 170 175
 Pro Leu Glu Lys Trp Gln Ile Asn Leu Asn Leu Phe Arg Thr Cys Met
 180 185 190
 Gly Tyr Ala Ile Pro Leu Ile Thr Ile Met Ile Cys Asn His Lys Val
 195 200 205
 Tyr Arg Ala Val Arg His Asn Gln Ala Thr Glu Asn Ser Glu Lys Arg
 210 215 220
 Arg Ile Ile Lys Leu Leu Ala Ser Ile Thr Leu Thr Phe Val Leu Cys
 225 230 235 240
 Phe Thr Pro Phe His Val Met Val Leu Ile Arg Cys Val Leu Glu Arg
 245 250 255
 Asp Met Asn Val Asn Asp Lys Ser Gly Trp Gln Thr Phe Thr Val Tyr
 260 265 270
 Arg Val Thr Val Ala Leu Thr Ser Leu Asn Cys Val Ala Asp Pro Ile
 275 280 285
 Leu Tyr Cys Phe Val Thr Glu Thr Gly Arg Ala Asp Met Trp Asn Ile
 290 295 300
 Leu Lys Leu Cys Thr Arg Lys His Asn Arg His Gln Gly Lys Lys Arg
 305 310 315 320
 Asp Ile Leu Ser Val Ser Thr Arg Asp Ala Val Glu Leu Glu Ile Ile
 325 330 335

Asp

<210> 10
 <211> 281
 <212> PRT
 <213> Mus musculus

<400> 10

Met Phe Ser Gly Leu Thr Leu Asn Cys Val Leu Leu Leu Leu Gln Leu
 1 5 10 15

Leu Leu Ala Arg Ser Leu Glu Asp Gly Tyr Lys Val Glu Val Gly Lys
 20 25 30
 Asn Ala Tyr Leu Pro Cys Ser Tyr Thr Leu Pro Thr Ser Gly Thr Leu
 35 40 45
 Val Pro Met Cys Trp Gly Lys Gly Phe Cys Pro Trp Ser Gln Cys Thr
 50 55 60
 Asn Glu Leu Leu Arg Thr Asp Glu Arg Asn Val Thr Tyr Gln Lys Ser
 65 70 75 80
 Ser Arg Tyr Gln Leu Lys Gly Asp Leu Asn Lys Gly Asp Val Ser Leu
 85 90 95
 Ile Ile Lys Asn Val Thr Leu Asp Asp His Gly Thr Tyr Cys Cys Arg
 100 105 110
 Ile Gln Phe Pro Gly Leu Met Asn Asp Lys Lys Leu Glu Leu Lys Leu
 115 120 125
 Asp Ile Lys Ala Ala Lys Val Thr Pro Ala Gln Thr Ala His Gly Asp
 130 135 140
 Ser Thr Thr Ala Ser Pro Arg Thr Leu Thr Thr Glu Arg Asn Gly Ser
 145 150 155 160
 Glu Thr Gln Thr Leu Val Thr Leu His Asn Asn Asn Gly Thr Lys Ile
 165 170 175
 Ser Thr Trp Ala Asp Glu Ile Lys Asp Ser Gly Glu Thr Ile Arg Thr
 180 185 190
 Ala Ile His Ile Gly Val Gly Val Ser Ala Gly Leu Thr Leu Ala Leu
 195 200 205
 Ile Ile Gly Val Leu Ile Leu Lys Trp Tyr Ser Cys Lys Lys Lys Lys
 210 215 220
 Leu Ser Ser Leu Ser Leu Ile Thr Leu Ala Asn Leu Pro Pro Gly Gly
 225 230 235 240
 Leu Ala Asn Ala Gly Ala Val Arg Ile Arg Ser Glu Glu Asn Ile Tyr
 245 250 255
 Thr Ile Glu Glu Asn Val Tyr Glu Val Glu Asn Ser Asn Glu Tyr Tyr
 260 265 270
 Cys Tyr Val Asn Ser Gln Gln Pro Ser
 275 280

<210> 11
 <211> 1257
 <212> DNA
 <213> Mus musculus

<220>

<221> CDS

<222> (22)..(1137)

<400> 11

```

ccgggtcgac ccacgcgtcc g atg aca ctg act gcc cac ctc tcc tac ttt 51
                        Met Thr Leu Thr Ala His Leu Ser Tyr Phe
                        1                      5                      10

ctg gtc ctg ttg tta gcg ggc caa ggc ctc agt gac tcc ctc ctc acc 99
Leu Val Leu Leu Leu Ala Gly Gln Gly Leu Ser Asp Ser Leu Leu Thr
                        15                      20                      25

aag gat gca ggt ccc cgc cca ctg gag ctg aag gaa gtc ttc aag ctg 147
Lys Asp Ala Gly Pro Arg Pro Leu Glu Leu Lys Glu Val Phe Lys Leu
                        30                      35                      40

ttc cag atc cgg ttc aac cgg agt tac tgg aac cca gca gag tac act 195
Phe Gln Ile Arg Phe Asn Arg Ser Tyr Trp Asn Pro Ala Glu Tyr Thr
                        45                      50                      55

cgc cgt ctg agc atc ttt gcc cac aat ctg gct cag gct caa agg cta 243
Arg Arg Leu Ser Ile Phe Ala His Asn Leu Ala Gln Ala Gln Arg Leu
                        60                      65                      70

cag caa gaa gac ttg ggt aca gct gag ttt gga gag act cca ttc agt 291
Gln Gln Glu Asp Leu Gly Thr Ala Glu Phe Gly Glu Thr Pro Phe Ser
                        75                      80                      85                      90

gac ctc aca gag gag gag ttt ggc cag tta tac ggg cag gag agg tca 339
Asp Leu Thr Glu Glu Glu Phe Gly Gln Leu Tyr Gly Gln Glu Arg Ser
                        95                      100                      105

cca gaa agg acc ccc aac atg acc aaa aag gta gag tct aac acg tgg 387
Pro Glu Arg Thr Pro Asn Met Thr Lys Lys Val Glu Ser Asn Thr Trp
                        110                      115                      120

ggg gaa tct gtg ccc cgc acc tgt gac tgg cgt aaa gca aag aac atc 435
Gly Glu Ser Val Pro Arg Thr Cys Asp Trp Arg Lys Ala Lys Asn Ile
                        125                      130                      135

atc tcg tcg gtc aag aac cag gga agc tgc aaa tgc tgc tgg gcc atg 483
Ile Ser Ser Val Lys Asn Gln Gly Ser Cys Lys Cys Cys Trp Ala Met
                        140                      145                      150

gca gct gcc gac aac atc cag gct ctg tgg cgc atc aaa cac cag cag 531
Ala Ala Ala Asp Asn Ile Gln Ala Leu Trp Arg Ile Lys His Gln Gln
                        155                      160                      165                      170

ttt gtg gac gtc tct gtg cag gag ctg ctg gac tgc gaa cgc tgt gga 579
Phe Val Asp Val Ser Val Gln Glu Leu Leu Asp Cys Glu Arg Cys Gly
                        175                      180                      185

aat ggt tgc aat ggt ggc ttc gtg tgg gac gca tat cta act gtc ctc 627
Asn Gly Cys Asn Gly Gly Phe Val Trp Asp Ala Tyr Leu Thr Val Leu
                        190                      195                      200

```

aac aac agt ggc ctg gcc agt gaa aag gat tat cca ttc cag ggg gac 675
 Asn Asn Ser Gly Leu Ala Ser Glu Lys Asp Tyr Pro Phe Gln Gly Asp
 205 210 215

aga aag cct cac aga tgc cta gcc aag aag tac aag aag gtg gcc tgg 723
 Arg Lys Pro His Arg Cys Leu Ala Lys Lys Tyr Lys Lys Val Ala Trp
 220 225 230

atc cag gat ttc acc atg ttg tcc aat aat gag cag gca att gcc cac 771
 Ile Gln Asp Phe Thr Met Leu Ser Asn Asn Glu Gln Ala Ile Ala His
 235 240 245 250

tac ctg gcc gtg cat gga cct atc acc gtg acc atc aac atg aaa cta 819
 Tyr Leu Ala Val His Gly Pro Ile Thr Val Thr Ile Asn Met Lys Leu
 255 260 265

ctc cag cat tac cag aag ggt gtc atc aag gct aca ccc agc tcc tgt 867
 Leu Gln His Tyr Gln Lys Gly Val Ile Lys Ala Thr Pro Ser Ser Cys
 270 275 280

gac cct cgg caa gtg gac cac tct gtc ttg ctg gtg ggc ttt ggc aag 915
 Asp Pro Arg Gln Val Asp His Ser Val Leu Leu Val Gly Phe Gly Lys
 285 290 295

gag aaa gag ggc atg cag aca ggg aca gtc ttg tcc cat tct cga aaa 963
 Glu Lys Glu Gly Met Gln Thr Gly Thr Val Leu Ser His Ser Arg Lys
 300 305 310

cgt cgc cac tcc tcc cca tac tgg atc ctg aag aac tcc tgg gga gct 1011
 Arg Arg His Ser Ser Pro Tyr Trp Ile Leu Lys Asn Ser Trp Gly Ala
 315 320 325 330

cac tgg ggc gag aag ggt tac ttc agg ctg tat cgg gga aac aac acc 1059
 His Trp Gly Glu Lys Gly Tyr Phe Arg Leu Tyr Arg Gly Asn Asn Thr
 335 340 345

tgt gga gtc acc aag tat ccc ttc aca gct caa gtg gac tca cca gta 1107
 Cys Gly Val Thr Lys Tyr Pro Phe Thr Ala Gln Val Asp Ser Pro Val
 350 355 360

aag aag gca cgg acc tct tgt cct ccc tga aggcagcagv cactcttctg 1157
 Lys Lys Ala Arg Thr Ser Cys Pro Pro
 365 370

cttctccac atggccactg ccccttgta gccctgccca catcctctct gtatggcttc 1217

ataaaccaag actgctccgt gaaaaaaaaa aaaaaaaaaa 1257

<210> 12

<211> 371

<212> PRT

<213> Mus musculus

<400> 12

Met Thr Leu Thr Ala His Leu Ser Tyr Phe Leu Val Leu Leu Leu Ala
 1 5 10 15

Gly Gln Gly Leu Ser Asp Ser Leu Leu Thr Lys Asp Ala Gly Pro Arg
 20 25 30
 Pro Leu Glu Leu Lys Glu Val Phe Lys Leu Phe Gln Ile Arg Phe Asn
 35 40 45
 Arg Ser Tyr Trp Asn Pro Ala Glu Tyr Thr Arg Arg Leu Ser Ile Phe
 50 55 60
 Ala His Asn Leu Ala Gln Ala Gln Arg Leu Gln Gln Glu Asp Leu Gly
 65 70 75 80
 Thr Ala Glu Phe Gly Glu Thr Pro Phe Ser Asp Leu Thr Glu Glu Glu
 85 90 95
 Phe Gly Gln Leu Tyr Gly Gln Glu Arg Ser Pro Glu Arg Thr Pro Asn
 100 105 110
 Met Thr Lys Lys Val Glu Ser Asn Thr Trp Gly Glu Ser Val Pro Arg
 115 120 125
 Thr Cys Asp Trp Arg Lys Ala Lys Asn Ile Ile Ser Ser Val Lys Asn
 130 135 140
 Gln Gly Ser Cys Lys Cys Cys Trp Ala Met Ala Ala Ala Asp Asn Ile
 145 150 155 160
 Gln Ala Leu Trp Arg Ile Lys His Gln Gln Phe Val Asp Val Ser Val
 165 170 175
 Gln Glu Leu Leu Asp Cys Glu Arg Cys Gly Asn Gly Cys Asn Gly Gly
 180 185 190
 Phe Val Trp Asp Ala Tyr Leu Thr Val Leu Asn Asn Ser Gly Leu Ala
 195 200 205
 Ser Glu Lys Asp Tyr Pro Phe Gln Gly Asp Arg Lys Pro His Arg Cys
 210 215 220
 Leu Ala Lys Lys Tyr Lys Lys Val Ala Trp Ile Gln Asp Phe Thr Met
 225 230 235 240
 Leu Ser Asn Asn Glu Gln Ala Ile Ala His Tyr Leu Ala Val His Gly
 245 250 255
 Pro Ile Thr Val Thr Ile Asn Met Lys Leu Leu Gln His Tyr Gln Lys
 260 265 270
 Gly Val Ile Lys Ala Thr Pro Ser Ser Cys Asp Pro Arg Gln Val Asp
 275 280 285
 His Ser Val Leu Leu Val Gly Phe Gly Lys Glu Lys Glu Gly Met Gln
 290 295 300
 Thr Gly Thr Val Leu Ser His Ser Arg Lys Arg Arg His Ser Ser Pro
 305 310 315 320

Tyr Trp Ile Leu Lys Asn Ser Trp Gly Ala His Trp Gly Glu Lys Gly
 325 330 335

Tyr Phe Arg Leu Tyr Arg Gly Asn Asn Thr Cys Gly Val Thr Lys Tyr
 340 345 350

Pro Phe Thr Ala Gln Val Asp Ser Pro Val Lys Lys Ala Arg Thr Ser
 355 360 365

Cys Pro Pro
 370

<210> 13

<211> 130

<212> PRT

<213> Mus musculus

<400> 13

Met Arg Gln Lys Ala Val Ser Leu Phe Leu Cys Tyr Leu Leu Leu Phe
 1 5 10 15

Thr Cys Ser Gly Val Glu Ala Gly Lys Lys Lys Cys Ser Glu Ser Ser
 20 25 30

Asp Ser Gly Ser Gly Phe Trp Lys Ala Leu Thr Phe Met Ala Val Gly
 35 40 45

Gly Gly Leu Ala Val Ala Gly Leu Pro Ala Leu Gly Phe Thr Gly Ala
 50 55 60

Gly Ile Ala Ala Asn Ser Val Ala Ala Ser Leu Met Ser Trp Ser Ala
 65 70 75 80

Ile Leu Asn Gly Gly Gly Val Pro Ala Gly Gly Leu Val Ala Thr Leu
 85 90 95

Gln Ser Leu Gly Ala Gly Gly Ser Ser Val Ile Thr Gly Asn Ile Gly
 100 105 110

Ala Leu Met Gly Tyr Ala Thr His Lys Tyr Leu Asp Ser Glu Glu Asp
 115 120 125

Glu Glu
 130

<210> 14

<211> 130

<212> PRT

<213> Mus musculus

<400> 14

Met Arg Gln Lys Ala Val Ser Val Phe Leu Cys Tyr Leu Leu Leu Phe
 1 5 10 15

Thr Cys Ser Gly Val Glu Ala Gly Lys Lys Lys Cys Ser Glu Ser Ser
 20 25 30

Asp Ser Gly Ser Gly Phe Trp Lys Ala Leu Thr Phe Met Ala Val Gly
 35 40 45
 Gly Gly Leu Ala Val Ala Gly Leu Pro Ala Leu Gly Phe Thr Gly Ala
 50 55 60
 Gly Ile Ala Ala Asn Ser Val Ala Ala Ser Leu Met Ser Trp Ser Ala
 65 70 75 80
 Ile Leu Asn Gly Gly Gly Val Pro Ala Gly Gly Leu Val Ala Thr Leu
 85 90 95
 Gln Ser Leu Gly Ala Gly Gly Ser Ser Val Val Ile Gly Asn Ile Gly
 100 105 110
 Ala Leu Met Arg Tyr Ala Thr His Lys Tyr Leu Asp Ser Glu Glu Asp
 115 120 125
 Glu Glu
 130

<210> 15
 <211> 110
 <212> PRT
 <213> Mus musculus

<400> 15
 Val Glu Ala Gly Lys Lys Lys Cys Ser Glu Ser Ser Asp Ser Gly Ser
 1 5 10 15
 Gly Phe Trp Lys Ala Leu Thr Phe Met Ala Val Gly Gly Gly Leu Ala
 20 25 30
 Val Ala Gly Leu Pro Ala Leu Gly Phe Thr Gly Ala Gly Ile Ala Ala
 35 40 45
 Asn Ser Val Ala Ala Ser Leu Met Ser Trp Ser Ala Ile Leu Asn Gly
 50 55 60
 Gly Gly Val Pro Ala Gly Gly Leu Val Ala Thr Leu Gln Ser Leu Gly
 65 70 75 80
 Ala Gly Gly Ser Ser Val Val Ile Gly Asn Ile Gly Ala Leu Met Gly
 85 90 95
 Tyr Ala Thr His Lys Tyr Leu Asp Ser Glu Glu Asp Glu Glu
 100 105 110

<210> 16
 <211> 107
 <212> PRT
 <213> Mus musculus

<400> 16
 Gly Lys Lys Lys Cys Ser Glu Ser Ser Asp Ser Gly Ser Gly Phe Trp
 1 5 10 15

Lys Ala Leu Thr Phe Met Ala Val Gly Gly Gly Leu Ala Val Ala Gly
 20 25 30
 Leu Pro Ala Leu Gly Phe Thr Gly Ala Gly Ile Ala Ala Asn Ser Val
 35 40 45
 Ala Ala Ser Leu Met Ser Trp Ser Ala Ile Leu Asn Gly Gly Gly Val
 50 55 60
 Pro Ala Gly Gly Leu Val Ala Thr Leu Gln Ser Leu Gly Ala Gly Gly
 65 70 75 80
 Ser Ser Val Val Ile Gly Asn Ile Gly Ala Leu Met Gly Tyr Ala Thr
 85 90 95
 His Lys Tyr Leu Asp Ser Glu Glu Asp Glu Glu
 100 105

<210> 17
 <211> 122
 <212> PRT
 <213> Mus musculus

<400> 17
 Met Glu Ala Ser Ala Leu Thr Ser Ser Ala Val Thr Ser Val Ala Lys
 1 5 10 15
 Val Val Arg Val Ala Ser Gly Ser Ala Val Val Leu Pro Leu Ala Arg
 20 25 30
 Ile Ala Thr Val Val Ile Gly Gly Val Val Ala Met Ala Ala Val Pro
 35 40 45
 Met Val Leu Ser Ala Met Gly Phe Thr Ala Ala Gly Ile Ala Ser Ser
 50 55 60
 Ser Ile Ala Ala Lys Met Met Ser Ala Ala Ala Ile Ala Asn Gly Gly
 65 70 75 80
 Gly Val Ala Ser Gly Ser Leu Val Gly Thr Leu Gln Ser Leu Gly Ala
 85 90 95
 Thr Gly Leu Ser Gly Leu Thr Lys Phe Ile Leu Gly Ser Ile Gly Ser
 100 105 110
 Ala Ile Ala Ala Val Ile Ala Arg Phe Tyr
 115 120

<210> 18
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: primer

<400> 18

ttgccataga gagacctc

18

<210> 19

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 19

tgctgtccaa ttatacagg

19

<210> 20

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 20

gaacacggca ttgtcactaa ct

22

<210> 21

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 21

cctcatagat gggcactgtg t

21

<210> 22

<211> 843

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 22

atgttttcag gtcttaccct caactgtgtc ctgctgctgc tgcaactact acttgcaagg 60

tcattggaag atggttataa ggttgaggtt ggtaaaaatg cctatctgcc ctgcagttac 120

actctaccta catctgggac acttgtgcct atgtgctggg gcaagggatt ctgtccttgg 180

tcacagtgtg ccaatgagtt gtcagaact gatgaaagaa atgtgacata tcagaaatcc 240

agcagatacc agctaaaggg cgatctcaac aaaggagatg tgtctctgat cataaagaat 300

gtgactctgg atgaccatgg gacctactgc tgcaggatac agttccctgg tcttatgaat 360
 gataaaaaat tagaactgaa attagacatc aaagcagcca aggtcactcc agctcagact 420
 gcccattggg actctactac agcttctcca agaaccctaa ccacggagag aaatgggttca 480
 gagacacaga cactgggtgac cctccataat aacaatggaa caaaaatttc cacatggggt 540
 gatgaaatta aggactctgg agaaacgac agaactgcta tccacattgg agtgggagtc 600
 tctgctgggt tgaccctggc acttatcatt ggtgtcttaa tccttaaatg gtattcctgt 660
 aagaaaaaga agttatcgag tttgagcctt attacactgg ccaactggc tccaggaggg 720
 ttggcaaatg caggagcagt caggattcgc tctgaggaaa atatctacac catcgaggag 780
 aacgtatatg aagtggagaa ttcaaagag tactactgct acgtcaacag ccagcagcca 840
 tcc 843

<210> 23
 <211> 2236
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (42) .. (944)

<400> 23
 cgctaacaga ggtgtcctct gacttttctt ctgcaagctc c atg ttt tca cat ctt 56
 Met Phe Ser His Leu
 1 5

ccc ttt gac tgt gtc ctg ctg ctg ctg ctg cta cta ctt aca agg tcc 104
 Pro Phe Asp Cys Val Leu Leu Leu Leu Leu Leu Leu Leu Thr Arg Ser
 10 15 20

tca gaa gtg gaa tac aga gcg gag gtc ggt cag aat gcc tat ctg ccc 152
 Ser Glu Val Glu Tyr Arg Ala Glu Val Gly Gln Asn Ala Tyr Leu Pro
 25 30 35

tgc ttc tac acc cca gcc gcc cca ggg aac ctc gtg ccc gtc tgc tgg 200
 Cys Phe Tyr Thr Pro Ala Ala Pro Gly Asn Leu Val Pro Val Cys Trp
 40 45 50

ggc aaa gga gcc tgt cct gtg ttt gaa tgt ggc aac gtg gtg ctc agg 248
 Gly Lys Gly Ala Cys Pro Val Phe Glu Cys Gly Asn Val Val Leu Arg
 55 60 65

act gat gaa agg gat gtg aat tat tgg aca tcc aga tac tgg cta aat 296
 Thr Asp Glu Arg Asp Val Asn Tyr Trp Thr Ser Arg Tyr Trp Leu Asn
 70 75 80 85

ggg gat ttc cgc aaa gga gat gtg tcc ctg acc ata gag aat gtg act 344
 Gly Asp Phe Arg Lys Gly Asp Val Ser Leu Thr Ile Glu Asn Val Thr
 90 95 100

cta gca gac agt ggg atc tac tgc tgc cgg atc caa atc cca ggc ata 392
 Leu Ala Asp Ser Gly Ile Tyr Cys Cys Arg Ile Gln Ile Pro Gly Ile
 105 110 115

atg aat gat gaa aaa ttt aac ctg aag ttg gtc atc aaa cca gcc aag 440
 Met Asn Asp Glu Lys Phe Asn Leu Lys Leu Val Ile Lys Pro Ala Lys
 120 125 130

gtc acc cct gca ccg act ctg cag aga gac ttc act gca gcc ttt cca 488
 Val Thr Pro Ala Pro Thr Leu Gln Arg Asp Phe Thr Ala Ala Phe Pro
 135 140 145

agg atg ctt acc acc agg gga cat ggc cca gca gag aca cag aca ctg 536
 Arg Met Leu Thr Thr Arg Gly His Gly Pro Ala Glu Thr Gln Thr Leu
 150 155 160 165

ggg agc ctc cct gat ata aat cta aca caa ata tcc aca ttg gcc aat 584
 Gly Ser Leu Pro Asp Ile Asn Leu Thr Gln Ile Ser Thr Leu Ala Asn
 170 175 180

gag tta cgg gac tct aga ttg gcc aat gac tta cgg gac tct gga gca 632
 Glu Leu Arg Asp Ser Arg Leu Ala Asn Asp Leu Arg Asp Ser Gly Ala
 185 190 195

acc atc aga ata ggc atc tac atc gga gca ggg atc tgt gct ggg ctg 680
 Thr Ile Arg Ile Gly Ile Tyr Ile Gly Ala Gly Ile Cys Ala Gly Leu
 200 205 210

gct ctg gct ctt atc ttc ggc gct tta att ttc aaa tgg tat tct cat 728
 Ala Leu Ala Leu Ile Phe Gly Ala Leu Ile Phe Lys Trp Tyr Ser His
 215 220 225

agc aaa gag aag ata cag aat tta agc ctc atc tct ttg gcc aac ctc 776
 Ser Lys Glu Lys Ile Gln Asn Leu Ser Leu Ile Ser Leu Ala Asn Leu
 230 235 240 245

cct ccc tca gga ttg gca aat gca gta gca gag gga att cgc tca gaa 824
 Pro Pro Ser Gly Leu Ala Asn Ala Val Ala Glu Gly Ile Arg Ser Glu
 250 255 260

gaa aac atc tat acc att gaa gag aac gta tat gaa gtg gag gag ccc 872
 Glu Asn Ile Tyr Thr Ile Glu Glu Asn Val Tyr Glu Val Glu Glu Pro
 265 270 275

aat gag tat tat tgc tat gtc agc agc agg cag caa ccc tca caa cct 920
 Asn Glu Tyr Tyr Cys Tyr Val Ser Ser Arg Gln Gln Pro Ser Gln Pro
 280 285 290

ttg ggt tgt cgc ttt gca atg cca tagatccaac caccttattt ttgagcttgg 974
 Leu Gly Cys Arg Phe Ala Met Pro
 295 300

tgttttgtct ttttcagaaa ctatgagctg tgtcacctga ctggttttgg aggtttctgtc 1034
 cactgctatg gagcagagtt ttcccathtt cagaagataa tgactcacat gggaattgaa 1094
 ctgggacctg cactgaactt aaacaggcat gtcattgcct ctgtatttaa gccaacagag 1154

ttacccaacc cagagactgt taatcatgga tgtagagct caaacgggct tttatatata 1214
 ctaggaattc ttgacgtggg gtctctggag ctccaggaaa ttcgggcaca tcatatgtcc 1274
 atgaaacttc agataaacta ggrraaaactg ggtgctgagg tgaaagcata acttttttgg 1334
 cacagaaagt ctaaaggggc cactgatttt caaagagatc tgtgatccct ttttgttttt 1394
 tgtttttgag atggagtctt gctctgttgc ccaggctgga gtgcaatggc acaatctcgg 1454
 ctactgcaa gctccgctc ctgggttcaa gcgattctcc tgcctcagcc tcctgagtgg 1514
 ctgggattac aggcattcac caccatgccc agctaatttg ttgtattttt agtagagaca 1574
 gggtttcacc atgttgcca gtgtggtctc aaactcctga cctcatgatt tgcctgcctc 1634
 ggcctcccaa agcactggga ttacaggcgt gagccaccac atccagccag tgatccttaa 1694
 aagattaaga gatgactgga ctaggcttac cttgatcttg aagattccct tggaatgttg 1754
 agatttaggc ttatttgagc actacctgcc caactgtcag tgccagtgc tagcccttct 1814
 tttgtctccc ttatgaagac tgcctgcag ggctgagatg tggcaggagc tcccaggga 1874
 aaaggaagtg catttgattg gtgtgtattg gccaaagttt gcttggttg tgcttgaaag 1934
 aaaatatctc tgaccaactt ctgtattcgt ggaccaaact gaagctatat ttttcacaga 1994
 agaagaagca gtgacgggga cacaattct gttgcctggt ggaaagaagg caaaggcctt 2054
 cagcaatcta tattaccagc gctggatcct ttgacagaga gtggtcccta aacttaaatt 2114
 tcaagacggg ataggcttga tctgtcttgc ttattgttgc cccctgcgcc tagcacaatt 2174
 ctgacacaca attggaactt actaaaaatt tttttttact gttaaaaaaa aaaaaaaaaa 2234
 aa 2236

<210> 24
 <211> 301
 <212> PRT
 <213> Homo sapiens

<400> 24
 Met Phe Ser His Leu Pro Phe Asp Cys Val Leu Leu Leu Leu Leu Leu
 1 5 10 15
 Leu Leu Thr Arg Ser Ser Glu Val Glu Tyr Arg Ala Glu Val Gly Gln
 20 25 30
 Asn Ala Tyr Leu Pro Cys Phe Tyr Thr Pro Ala Ala Pro Gly Asn Leu
 35 40 45
 Val Pro Val Cys Trp Gly Lys Gly Ala Cys Pro Val Phe Glu Cys Gly
 50 55 60

Asn Val Val Leu Arg Thr Asp Glu Arg Asp Val Asn Tyr Trp Thr Ser
 65 70 75 80
 Arg Tyr Trp Leu Asn Gly Asp Phe Arg Lys Gly Asp Val Ser Leu Thr
 85 90 95
 Ile Glu Asn Val Thr Leu Ala Asp Ser Gly Ile Tyr Cys Cys Arg Ile
 100 105 110
 Gln Ile Pro Gly Ile Met Asn Asp Glu Lys Phe Asn Leu Lys Leu Val
 115 120 125
 Ile Lys Pro Ala Lys Val Thr Pro Ala Pro Thr Leu Gln Arg Asp Phe
 130 135 140
 Thr Ala Ala Phe Pro Arg Met Leu Thr Thr Arg Gly His Gly Pro Ala
 145 150 155 160
 Glu Thr Gln Thr Leu Gly Ser Leu Pro Asp Ile Asn Leu Thr Gln Ile
 165 170 175
 Ser Thr Leu Ala Asn Glu Leu Arg Asp Ser Arg Leu Ala Asn Asp Leu
 180 185 190
 Arg Asp Ser Gly Ala Thr Ile Arg Ile Gly Ile Tyr Ile Gly Ala Gly
 195 200 205
 Ile Cys Ala Gly Leu Ala Leu Ala Leu Ile Phe Gly Ala Leu Ile Phe
 210 215 220
 Lys Trp Tyr Ser His Ser Lys Glu Lys Ile Gln Asn Leu Ser Leu Ile
 225 230 235 240
 Ser Leu Ala Asn Leu Pro Pro Ser Gly Leu Ala Asn Ala Val Ala Glu
 245 250 255
 Gly Ile Arg Ser Glu Glu Asn Ile Tyr Thr Ile Glu Glu Asn Val Tyr
 260 265 270
 Glu Val Glu Glu Pro Asn Glu Tyr Tyr Cys Tyr Val Ser Ser Arg Gln
 275 280 285
 Gln Pro Ser Gln Pro Leu Gly Cys Arg Phe Ala Met Pro
 290 295 300

<210> 25

<211> 37

<212> DNA

<213> Artificial Sequence

<220>

 <223> Description of Artificial Sequence:
 oligonucleotide

<400> 25

aaatttattc tcgaggaccc acgcgtccgg atttccc

37

<210> 26
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide oligonucleotide

<400> 26
ttaatttgga tccccagttc tgatcgtttc tccagagtc

39

<210> 27
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 27
aaattttattc tcgagcgcta acagaggtgt cc

32

<210> 28
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 28
ttaatttgga tcccctctga tggttgctcc agagtcccg

39

<210> 29
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 29
ccgcgggtac cagtaaactcg tcctgggggtg g

31

<210> 30
<211> 36
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
oligonucleotide

<400> 30

aaataaagga tccctacatc cagcaactat gtagta

36

<210> 31

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
oligonucleotide

<400> 31

gcgcaattga ctagtgaccc acgcgtccgg atttc

35

<210> 32

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
oligonucleotide

<400> 32

gacgcggatc ctcaggatgg ctgctggctg

30

<210> 33

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
oligonucleotide

<400> 33

gaacacacta gtactatcct gtgccattgc catagaga

38

<210> 34

<211> 44

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
oligonucleotide

<400> 34

ggaatattgg gcccttgat cccaagtctg cacacctgca ctcc

44

<210> 35
 <211> 21
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 oligonucleotide

<400> 35
 gtaaatacgtc ctgggggtctg g 21

<210> 36
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 oligonucleotide

<400> 36
 ccttctgata acacaagcat aaatc 25

<210> 37
 <211> 903
 <212> DNA
 <213> Homo sapiens

<400> 37
 atgttttcac atcttccctt tgactgtgtc ctgctgctgc tgctgctact acttacaagg 60
 tcctcagaag tggaatacag agcggagggtc ggtcagaatg cctatctgcc ctgcttctac 120
 accccagccg ccccagggaa cctcgtgccc gtctgctggg gcaaaggagc ctgtcctgtg 180
 tttgaatgtg gcaacgtggg gctcaggact gatgaaaggg atgtgaatta ttggacatcc 240
 agatactggc taaatgggga tttccgcaaa ggagatgtgt ccctgaccat agagaatgtg 300
 actctagcag acagtgggat ctactgtgtc cggatccaaa tcccaggcat aatgaatgat 360
 gaaaaattta acctgaagtt ggatcatcaa ccagccaagg tcaccctgc accgactctg 420
 cagagagact tcaactgcagc ctttccaagg atgcttacca ccaggggaca tggcccagca 480
 gagacacaga cactggggag cctccctgat ataaatctaa cacaaatc cacttggtgc 540
 aatgagttac gggactctag attggccaat gacttacggg actctggagc aaccatcaga 600
 ataggcatct acatcggagc agggatctgt gctgggctgg ctctggctct tatcttcggc 660
 gctttaattt tcaaattgga ttctcatagc aaagagaaga tacagaattt aagcctcatc 720
 tctttggcca acctccctcc ctcaggattg gcaaatgcag tagcagaggg aattcgtca 780

gaagaaaaca tctataccat tgaagagaac gtatatgaag tggaggagcc caatgagtat 840
 tattgctatg tcagcagcag gcagcaaccc tcacaacctt tgggttgctg ctttgcaatg 900
 cca 903

<210> 38
 <211> 1704
 <212> DNA
 <213> Mus musculus

<220>
 <221> CAAT_signal
 <222> (1)..(1704)

<220>
 <221> CDS
 <222> (1)..(1701)

<400> 38
 atg att gac aga cag aga atg gga ctt tgg gct ttg gca att ctg aca 48
 Met Ile Asp Arg Gln Arg Met Gly Leu Trp Ala Leu Ala Ile Leu Thr
 1 5 10 15
 ctt ccc atg tat ttg aca gtt acg gag ggc agt aaa tcg tcc tgg ggt 96
 Leu Pro Met Tyr Leu Thr Val Thr Glu Gly Ser Lys Ser Ser Trp Gly
 20 25 30
 ctg gaa aat gag gct tta att gtg aga tgc ccc caa aga gga cgc tcg 144
 Leu Glu Asn Glu Ala Leu Ile Val Arg Cys Pro Gln Arg Gly Arg Ser
 35 40 45
 act tat cct gtg gaa tgg tat tac tca gat aca aat gaa agt att cct 192
 Thr Tyr Pro Val Glu Trp Tyr Tyr Ser Asp Thr Asn Glu Ser Ile Pro
 50 55 60
 act caa aaa aga aat cgg atc ttt gtc tca aga gat cgt ctg aag ttt 240
 Thr Gln Lys Arg Asn Arg Ile Phe Val Ser Arg Asp Arg Leu Lys Phe
 65 70 75 80
 cta cca gcc aga gtg gaa gac tct ggg att tat gct tgt gtt atc aga 288
 Leu Pro Ala Arg Val Glu Asp Ser Gly Ile Tyr Ala Cys Val Ile Arg
 85 90 95
 agc ccc aac ttg aat aag act gga tac ttg aat gtc acc ata cat aaa 336
 Ser Pro Asn Leu Asn Lys Thr Gly Tyr Leu Asn Val Thr Ile His Lys
 100 105 110
 aag ccg cca agc tgc aat atc cct gat tat ttg atg tac tcg aca gta 384
 Lys Pro Pro Ser Cys Asn Ile Pro Asp Tyr Leu Met Tyr Ser Thr Val
 115 120 125
 cgt gga tca gat aaa aat ttc aag ata acg tgt cca aca att gac ctg 432
 Arg Gly Ser Asp Lys Asn Phe Lys Ile Thr Cys Pro Thr Ile Asp Leu
 130 135 140

tat aat tgg aca gca cct gtt cag tgg ttt aag aac tgc aaa gct ctc	480
Tyr Asn Trp Thr Ala Pro Val Gln Trp Phe Lys Asn Cys Lys Ala Leu	
145 150 155 160	
caa gag cca agg ttc agg gca cac agg tcc tac ttg ttc att gac aac	528
Gln Glu Pro Arg Phe Arg Ala His Arg Ser Tyr Leu Phe Ile Asp Asn	
165 170 175	
gtg act cat gat gat gaa ggt gac tac act tgt caa ttc aca cac gcg	576
Val Thr His Asp Asp Glu Gly Asp Tyr Thr Cys Gln Phe Thr His Ala	
180 185 190	
gag aat gga acc aac tac atc gtg acg gcc acc aga tca ttc aca gtt	624
Glu Asn Gly Thr Asn Tyr Ile Val Thr Ala Thr Arg Ser Phe Thr Val	
195 200 205	
gaa gaa aaa ggc ttt tct atg ttt cca gta att aca aat cct cca tac	672
Glu Glu Lys Gly Phe Ser Met Phe Pro Val Ile Thr Asn Pro Pro Tyr	
210 215 220	
aac cac aca atg gaa gtg gaa ata gga aaa cca gca agt att gcc tgt	720
Asn His Thr Met Glu Val Glu Ile Gly Lys Pro Ala Ser Ile Ala Cys	
225 230 235 240	
tca gct tgc ttt ggc aaa ggc tct cac ttc ttg gct gat gtc ctg tgg	768
Ser Ala Cys Phe Gly Lys Gly Ser His Phe Leu Ala Asp Val Leu Trp	
245 250 255	
cag att aac aaa aca gta gtt gga aat ttt ggt gaa gca aga att caa	816
Gln Ile Asn Lys Thr Val Val Gly Asn Phe Gly Glu Ala Arg Ile Gln	
260 265 270	
gaa gag gaa ggt cga aat gaa agt tcc agc aat gac atg gat tgt tta	864
Glu Glu Glu Gly Arg Asn Glu Ser Ser Ser Asn Asp Met Asp Cys Leu	
275 280 285	
acc tca gtg tta agg ata act ggt gtg aca gaa aag gac ctg tcc ctg	912
Thr Ser Val Leu Arg Ile Thr Gly Val Thr Glu Lys Asp Leu Ser Leu	
290 295 300	
gaa tat gac tgt ctg gcc ctg aac ctt cat ggc atg ata agg cac acc	960
Glu Tyr Asp Cys Leu Ala Leu Asn Leu His Gly Met Ile Arg His Thr	
305 310 315 320	
ata agg ctg aga agg aaa caa cca att gat cac cga agc atc tac tac	1008
Ile Arg Leu Arg Arg Lys Gln Pro Ile Asp His Arg Ser Ile Tyr Tyr	
325 330 335	
ata gtt gct gga tgt agt tta ttg cta atg ttt atc aat gtc ttg gtg	1056
Ile Val Ala Gly Cys Ser Leu Leu Leu Met Phe Ile Asn Val Leu Val	
340 345 350	
ata gtc tta aaa gtg ttc tgg att gag gtt gct ctg ttc tgg aga gat	1104
Ile Val Leu Lys Val Phe Trp Ile Glu Val Ala Leu Phe Trp Arg Asp	
355 360 365	

ata gtg aca cct tac aaa acc cgg aac gat ggc aag ctc tac gat gcg	1152
Ile Val Thr Pro Tyr Lys Thr Arg Asn Asp Gly Lys Leu Tyr Asp Ala	
370 375 380	
tac atc att tac cct cgg gtc ttc cgg ggc agc gcg gcg gga acc cac	1200
Tyr Ile Ile Tyr Pro Arg Val Phe Arg Gly Ser Ala Ala Gly Thr His	
385 390 395 400	
tct gtg gag tac ttt gtt cac cac act ctg ccc gac gtt ctt gaa aat	1248
Ser Val Glu Tyr Phe Val His His Thr Leu Pro Asp Val Leu Glu Asn	
405 410 415	
aaa tgt ggc tac aaa ttg tgc att tat ggg aga gac ctg tta cct ggg	1296
Lys Cys Gly Tyr Lys Leu Cys Ile Tyr Gly Arg Asp Leu Leu Pro Gly	
420 425 430	
caa gat gca gcc acc gtg gtg gaa agc agt atc cag aat agc aga aga	1344
Gln Asp Ala Ala Thr Val Val Glu Ser Ser Ile Gln Asn Ser Arg Arg	
435 440 445	
cag gtg ttt gtt ctg gcc cct cac atg atg cac agc aag gaa ttt gcc	1392
Gln Val Phe Val Leu Ala Pro His Met Met His Ser Lys Glu Phe Ala	
450 455 460	
tac gag cag gag att gct ctg cac agc gcc ctc atc cag aac aac tcc	1440
Tyr Glu Gln Glu Ile Ala Leu His Ser Ala Leu Ile Gln Asn Asn Ser	
465 470 475 480	
aag gtg att ctt att gaa atg gag cct ctg ggt gag gca agc cga cta	1488
Lys Val Ile Leu Ile Glu Met Glu Pro Leu Gly Glu Ala Ser Arg Leu	
485 490 495	
cag gtt ggg gac ctg caa gat tct ctc cag cat ctt gtg aaa att cag	1536
Gln Val Gly Asp Leu Gln Asp Ser Leu Gln His Leu Val Lys Ile Gln	
500 505 510	
ggg acc atc aag tgg agg gaa gat cat gtg gcc gac aag cag tct cta	1584
Gly Thr Ile Lys Trp Arg Glu Asp His Val Ala Asp Lys Gln Ser Leu	
515 520 525	
agt tcc aaa ttc tgg aag cat gtg agg tac caa atg cca gtg cca gaa	1632
Ser Ser Lys Phe Trp Lys His Val Arg Tyr Gln Met Pro Val Pro Glu	
530 535 540	
aga gcc tcc aag acg gca tct gtt gcg gct ccg ttg agt ggc aag gca	1680
Arg Ala Ser Lys Thr Ala Ser Val Ala Ala Pro Leu Ser Gly Lys Ala	
545 550 555 560	
tgc tta gac ctg aaa cac ttt tga	1704
Cys Leu Asp Leu Lys His Phe	
565	

<210> 39

<211> 567

<212> PRT

<213> Mus musculus

<400> 39

Met Ile Asp Arg Gln Arg Met Gly Leu Trp Ala Leu Ala Ile Leu Thr
 1 5 10 15
 Leu Pro Met Tyr Leu Thr Val Thr Glu Gly Ser Lys Ser Ser Trp Gly
 20 25 30
 Leu Glu Asn Glu Ala Leu Ile Val Arg Cys Pro Gln Arg Gly Arg Ser
 35 40 45
 Thr Tyr Pro Val Glu Trp Tyr Tyr Ser Asp Thr Asn Glu Ser Ile Pro
 50 55 60
 Thr Gln Lys Arg Asn Arg Ile Phe Val Ser Arg Asp Arg Leu Lys Phe
 65 70 75 80
 Leu Pro Ala Arg Val Glu Asp Ser Gly Ile Tyr Ala Cys Val Ile Arg
 85 90 95
 Ser Pro Asn Leu Asn Lys Thr Gly Tyr Leu Asn Val Thr Ile His Lys
 100 105 110
 Lys Pro Pro Ser Cys Asn Ile Pro Asp Tyr Leu Met Tyr Ser Thr Val
 115 120 125
 Arg Gly Ser Asp Lys Asn Phe Lys Ile Thr Cys Pro Thr Ile Asp Leu
 130 135 140
 Tyr Asn Trp Thr Ala Pro Val Gln Trp Phe Lys Asn Cys Lys Ala Leu
 145 150 155 160
 Gln Glu Pro Arg Phe Arg Ala His Arg Ser Tyr Leu Phe Ile Asp Asn
 165 170 175
 Val Thr His Asp Asp Glu Gly Asp Tyr Thr Cys Gln Phe Thr His Ala
 180 185 190
 Glu Asn Gly Thr Asn Tyr Ile Val Thr Ala Thr Arg Ser Phe Thr Val
 195 200 205
 Glu Glu Lys Gly Phe Ser Met Phe Pro Val Ile Thr Asn Pro Pro Tyr
 210 215 220
 Asn His Thr Met Glu Val Glu Ile Gly Lys Pro Ala Ser Ile Ala Cys
 225 230 235 240
 Ser Ala Cys Phe Gly Lys Gly Ser His Phe Leu Ala Asp Val Leu Trp
 245 250 255
 Gln Ile Asn Lys Thr Val Val Gly Asn Phe Gly Glu Ala Arg Ile Gln
 260 265 270
 Glu Glu Glu Gly Arg Asn Glu Ser Ser Ser Asn Asp Met Asp Cys Leu
 275 280 285
 Thr Ser Val Leu Arg Ile Thr Gly Val Thr Glu Lys Asp Leu Ser Leu
 290 295 300

Glu Tyr Asp Cys Leu Ala Leu Asn Leu His Gly Met Ile Arg His Thr
 305 310 315 320
 Ile Arg Leu Arg Arg Lys Gln Pro Ile Asp His Arg Ser Ile Tyr Tyr
 325 330 335
 Ile Val Ala Gly Cys Ser Leu Leu Leu Met Phe Ile Asn Val Leu Val
 340 345 350
 Ile Val Leu Lys Val Phe Trp Ile Glu Val Ala Leu Phe Trp Arg Asp
 355 360 365
 Ile Val Thr Pro Tyr Lys Thr Arg Asn Asp Gly Lys Leu Tyr Asp Ala
 370 375 380
 Tyr Ile Ile Tyr Pro Arg Val Phe Arg Gly Ser Ala Ala Gly Thr His
 385 390 395 400
 Ser Val Glu Tyr Phe Val His His Thr Leu Pro Asp Val Leu Glu Asn
 405 410 415
 Lys Cys Gly Tyr Lys Leu Cys Ile Tyr Gly Arg Asp Leu Leu Pro Gly
 420 425 430
 Gln Asp Ala Ala Thr Val Val Glu Ser Ser Ile Gln Asn Ser Arg Arg
 435 440 445
 Gln Val Phe Val Leu Ala Pro His Met Met His Ser Lys Glu Phe Ala
 450 455 460
 Tyr Glu Gln Glu Ile Ala Leu His Ser Ala Leu Ile Gln Asn Asn Ser
 465 470 475 480
 Lys Val Ile Leu Ile Glu Met Glu Pro Leu Gly Glu Ala Ser Arg Leu
 485 490 495
 Gln Val Gly Asp Leu Gln Asp Ser Leu Gln His Leu Val Lys Ile Gln
 500 505 510
 Gly Thr Ile Lys Trp Arg Glu Asp His Val Ala Asp Lys Gln Ser Leu
 515 520 525
 Ser Ser Lys Phe Trp Lys His Val Arg Tyr Gln Met Pro Val Pro Glu
 530 535 540
 Arg Ala Ser Lys Thr Ala Ser Val Ala Ala Pro Leu Ser Gly Lys Ala
 545 550 555 560
 Cys Leu Asp Leu Lys His Phe
 565

<210> 40
 <211> 1029
 <212> DNA
 <213> Mus musculus

<220>

<221> CDS

<222> (1) .. (1026)

<400> 40

atg att gac aga cag aga atg gga ctt tgg gct ttg gca att ctg aca	48
Met Ile Asp Arg Gln Arg Met Gly Leu Trp Ala Leu Ala Ile Leu Thr	
1 5 10 15	
ctt ccc atg tat ttg aca gtt acg gag ggc agt aaa tcg tcc tgg ggt	96
Leu Pro Met Tyr Leu Thr Val Thr Glu Gly Ser Lys Ser Ser Trp Gly	
20 25 30	
ctg gaa aat gag gct tta att gtg aga tgc ccc caa aga gga cgc tcg	144
Leu Glu Asn Glu Ala Leu Ile Val Arg Cys Pro Gln Arg Gly Arg Ser	
35 40 45	
act tat cct gtg gaa tgg tat tac tca gat aca aat gaa agt att cct	192
Thr Tyr Pro Val Glu Trp Tyr Tyr Ser Asp Thr Asn Glu Ser Ile Pro	
50 55 60	
act caa aaa aga aat cgg atc ttt gtc tca aga gat cgt ctg aag ttt	240
Thr Gln Lys Arg Asn Arg Ile Phe Val Ser Arg Asp Arg Leu Lys Phe	
65 70 75 80	
cta cca gcc aga gtg gaa gac tct ggg att tat gct tgt gtt atc aga	288
Leu Pro Ala Arg Val Glu Asp Ser Gly Ile Tyr Ala Cys Val Ile Arg	
85 90 95	
agc ccc aac ttg aat aag act gga tac ttg aat gtc acc ata cat aaa	336
Ser Pro Asn Leu Asn Lys Thr Gly Tyr Leu Asn Val Thr Ile His Lys	
100 105 110	
aag ccg cca agc tgc aat atc cct gat tat ttg atg tac tcg aca gta	384
Lys Pro Pro Ser Cys Asn Ile Pro Asp Tyr Leu Met Tyr Ser Thr Val	
115 120 125	
cgt gga tca gat aaa aat ttc aag ata acg tgt cca aca att gac ctg	432
Arg Gly Ser Asp Lys Asn Phe Lys Ile Thr Cys Pro Thr Ile Asp Leu	
130 135 140	
tat aat tgg aca gca cct gtt cag tgg ttt aag aac tgc aaa gct ctc	480
Tyr Asn Trp Thr Ala Pro Val Gln Trp Phe Lys Asn Cys Lys Ala Leu	
145 150 155 160	
caa gag cca agg ttc agg gca cac agg tcc tac ttg ttc att gac aac	528
Gln Glu Pro Arg Phe Arg Ala His Arg Ser Tyr Leu Phe Ile Asp Asn	
165 170 175	
gtg act cat gat gat gaa ggt gac tac act tgt caa ttc aca cac gcg	576
Val Thr His Asp Asp Glu Gly Asp Tyr Thr Cys Gln Phe Thr His Ala	
180 185 190	
gag aat gga acc aac tac atc gtg acg gcc acc aga tca ttc aca gtt	624
Glu Asn Gly Thr Asn Tyr Ile Val Thr Ala Thr Arg Ser Phe Thr Val	
195 200 205	

gaa gaa aaa ggc ttt tct atg ttt cca gta att aca aat cct cca tac 672
 Glu Glu Lys Gly Phe Ser Met Phe Pro Val Ile Thr Asn Pro Pro Tyr
 210 215 220

aac cac aca atg gaa gtg gaa ata gga aaa cca gca agt att gcc tgt 720
 Asn His Thr Met Glu Val Glu Ile Gly Lys Pro Ala Ser Ile Ala Cys
 225 230 235 240

tca gct tgc ttt ggc aaa ggc tct cac ttc ttg gct gat gtc ctg tgg 768
 Ser Ala Cys Phe Gly Lys Gly Ser His Phe Leu Ala Asp Val Leu Trp
 245 250 255

cag att aac aaa aca gta gtt gga aat ttt ggt gaa gca aga att caa 816
 Gln Ile Asn Lys Thr Val Val Gly Asn Phe Gly Glu Ala Arg Ile Gln
 260 265 270

gaa gag gaa ggt cga aat gaa agt tcc agc aat gac atg gat tgt tta 864
 Glu Glu Glu Gly Arg Asn Glu Ser Ser Ser Asn Asp Met Asp Cys Leu
 275 280 285

acc tca gtg tta agg ata act ggt gtg aca gaa aag gac ctg tcc ctg 912
 Thr Ser Val Leu Arg Ile Thr Gly Val Thr Glu Lys Asp Leu Ser Leu
 290 295 300

gaa tat gac tgt ctg gcc ctg aac ctt cat ggc atg ata agg cac acc 960
 Glu Tyr Asp Cys Leu Ala Leu Asn Leu His Gly Met Ile Arg His Thr
 305 310 315 320

ata agg ctg aga agg aaa caa cca att gat cac cga agc atc tac tac 1008
 Ile Arg Leu Arg Arg Lys Gln Pro Ile Asp His Arg Ser Ile Tyr Tyr
 325 330 335

ata gtt gct gga tgt agt tga 1029
 Ile Val Ala Gly Cys Ser
 340

<210> 41

<211> 342

<212> PRT

<213> Mus musculus

<400> 41

Met Ile Asp Arg Gln Arg Met Gly Leu Trp Ala Leu Ala Ile Leu Thr
 1 5 10 15

Leu Pro Met Tyr Leu Thr Val Thr Glu Gly Ser Lys Ser Ser Trp Gly
 20 25 30

Leu Glu Asn Glu Ala Leu Ile Val Arg Cys Pro Gln Arg Gly Arg Ser
 35 40 45

Thr Tyr Pro Val Glu Trp Tyr Tyr Ser Asp Thr Asn Glu Ser Ile Pro
 50 55 60

Thr Gln Lys Arg Asn Arg Ile Phe Val Ser Arg Asp Arg Leu Lys Phe
 65 70 75 80

Leu Pro Ala Arg Val Glu Asp Ser Gly Ile Tyr Ala Cys Val Ile Arg
 85 90 95
 Ser Pro Asn Leu Asn Lys Thr Gly Tyr Leu Asn Val Thr Ile His Lys
 100 105 110
 Lys Pro Pro Ser Cys Asn Ile Pro Asp Tyr Leu Met Tyr Ser Thr Val
 115 120 125
 Arg Gly Ser Asp Lys Asn Phe Lys Ile Thr Cys Pro Thr Ile Asp Leu
 130 135 140
 Tyr Asn Trp Thr Ala Pro Val Gln Trp Phe Lys Asn Cys Lys Ala Leu
 145 150 155 160
 Gln Glu Pro Arg Phe Arg Ala His Arg Ser Tyr Leu Phe Ile Asp Asn
 165 170 175
 Val Thr His Asp Asp Glu Gly Asp Tyr Thr Cys Gln Phe Thr His Ala
 180 185 190
 Glu Asn Gly Thr Asn Tyr Ile Val Thr Ala Thr Arg Ser Phe Thr Val
 195 200 205
 Glu Glu Lys Gly Phe Ser Met Phe Pro Val Ile Thr Asn Pro Pro Tyr
 210 215 220
 Asn His Thr Met Glu Val Glu Ile Gly Lys Pro Ala Ser Ile Ala Cys
 225 230 235 240
 Ser Ala Cys Phe Gly Lys Gly Ser His Phe Leu Ala Asp Val Leu Trp
 245 250 255
 Gln Ile Asn Lys Thr Val Val Gly Asn Phe Gly Glu Ala Arg Ile Gln
 260 265 270
 Glu Glu Glu Gly Arg Asn Glu Ser Ser Ser Asn Asp Met Asp Cys Leu
 275 280 285
 Thr Ser Val Leu Arg Ile Thr Gly Val Thr Glu Lys Asp Leu Ser Leu
 290 295 300
 Glu Tyr Asp Cys Leu Ala Leu Asn Leu His Gly Met Ile Arg His Thr
 305 310 315 320
 Ile Arg Leu Arg Arg Lys Gln Pro Ile Asp His Arg Ser Ile Tyr Tyr
 325 330 335
 Ile Val Ala Gly Cys Ser
 340

<210> 42

<211> 606

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(603)

<400> 42

```

aga gat ata gtg aca cct tac aaa acc cgg aac gat ggc aag ctc tac   48
Arg Asp Ile Val Thr Pro Tyr Lys Thr Arg Asn Asp Gly Lys Leu Tyr
  1             5             10             15

gat gcg tac atc att tac cct cgg gtc ttc cgg ggc agc gcg gcg gga   96
Asp Ala Tyr Ile Ile Tyr Pro Arg Val Phe Arg Gly Ser Ala Ala Gly
             20             25             30

acc cac tct gtg gag tac ttt gtt cac cac act ctg ccc gac gtt ctt   144
Thr His Ser Val Glu Tyr Phe Val His His Thr Leu Pro Asp Val Leu
             35             40             45

gaa aat aaa tgt ggc tac aaa ttg tgc att tat ggg aga gac ctg tta   192
Glu Asn Lys Cys Gly Tyr Lys Leu Cys Ile Tyr Gly Arg Asp Leu Leu
             50             55             60

cct ggg caa gat gca gcc acc gtg gtg gaa agc agt atc cag aat agc   240
Pro Gly Gln Asp Ala Ala Thr Val Val Glu Ser Ser Ile Gln Asn Ser
             65             70             75             80

aga aga cag gtg ttt gtt ctg gcc cct cac atg atg cac agc aag gaa   288
Arg Arg Gln Val Phe Val Leu Ala Pro His Met Met His Ser Lys Glu
             85             90             95

ttt gcc tac gag cag gag att gct ctg cac agc gcc ctc atc cag aac   336
Phe Ala Tyr Glu Gln Glu Ile Ala Leu His Ser Ala Leu Ile Gln Asn
             100             105             110

aac tcc aag gtg att ctt att gaa atg gag cct ctg ggt gag gca agc   384
Asn Ser Lys Val Ile Leu Ile Glu Met Glu Pro Leu Gly Glu Ala Ser
             115             120             125

cga cta cag gtt ggg gac ctg caa gat tct ctc cag cat ctt gtg aaa   432
Arg Leu Gln Val Gly Asp Leu Gln Asp Ser Leu Gln His Leu Val Lys
             130             135             140

att cag ggg acc atc aag tgg agg gaa gat cat gtg gcc gac aag cag   480
Ile Gln Gly Thr Ile Lys Trp Arg Glu Asp His Val Ala Asp Lys Gln
             145             150             155             160

tct cta agt tcc aaa ttc tgg aag cat gtg agg tac caa atg cca gtg   528
Ser Leu Ser Ser Lys Phe Trp Lys His Val Arg Tyr Gln Met Pro Val
             165             170             175

cca gaa aga gcc tcc aag acg gca tct gtt gcg gct ccg ttg agt ggc   576
Pro Glu Arg Ala Ser Lys Thr Ala Ser Val Ala Ala Pro Leu Ser Gly
             180             185             190

aag gca tgc tta gac ctg aaa cac ttt tga   606
Lys Ala Cys Leu Asp Leu Lys His Phe
             195             200

```

<210> 43

<211> 201
 <212> PRT
 <213> Mus musculus

<400> 43
 Arg Asp Ile Val Thr Pro Tyr Lys Thr Arg Asn Asp Gly Lys Leu Tyr
 1 5 10 15
 Asp Ala Tyr Ile Ile Tyr Pro Arg Val Phe Arg Gly Ser Ala Ala Gly
 20 25 30
 Thr His Ser Val Glu Tyr Phe Val His His Thr Leu Pro Asp Val Leu
 35 40 45
 Glu Asn Lys Cys Gly Tyr Lys Leu Cys Ile Tyr Gly Arg Asp Leu Leu
 50 55 60
 Pro Gly Gln Asp Ala Ala Thr Val Val Glu Ser Ser Ile Gln Asn Ser
 65 70 75 80
 Arg Arg Gln Val Phe Val Leu Ala Pro His Met Met His Ser Lys Glu
 85 90 95
 Phe Ala Tyr Glu Gln Glu Ile Ala Leu His Ser Ala Leu Ile Gln Asn
 100 105 110
 Asn Ser Lys Val Ile Leu Ile Glu Met Glu Pro Leu Gly Glu Ala Ser
 115 120 125
 Arg Leu Gln Val Gly Asp Leu Gln Asp Ser Leu Gln His Leu Val Lys
 130 135 140
 Ile Gln Gly Thr Ile Lys Trp Arg Glu Asp His Val Ala Asp Lys Gln
 145 150 155 160
 Ser Leu Ser Ser Lys Phe Trp Lys His Val Arg Tyr Gln Met Pro Val
 165 170 175
 Pro Glu Arg Ala Ser Lys Thr Ala Ser Val Ala Ala Pro Leu Ser Gly
 180 185 190
 Lys Ala Cys Leu Asp Leu Lys His Phe
 195 200

<210> 44
 <211> 1357
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (47)..(1030)

<400> 44
 atctcaacaa cgagttacca atacttgctc ttgattgata aacaga atg ggg ttt 55
 Met Gly Phe
 1

tgg atc tta gca att ctc aca att ctc atg tat tcc aca gca gca aag	103
Trp Ile Leu Ala Ile Leu Thr Ile Leu Met Tyr Ser Thr Ala Ala Lys	
5 10 15	
ttt agt aaa caa tca tgg ggc ctg gaa aat gag gct tta att gta aga	151
Phe Ser Lys Gln Ser Trp Gly Leu Glu Asn Glu Ala Leu Ile Val Arg	
20 25 30 35	
tgt cct aga caa gga aaa cct agt tac acc gtg gat tgg tat tac tca	199
Cys Pro Arg Gln Gly Lys Pro Ser Tyr Thr Val Asp Trp Tyr Tyr Ser	
40 45 50	
caa aca aac aaa agt att ccc act cag gaa aga aat cgt gtg ttt gcc	247
Gln Thr Asn Lys Ser Ile Pro Thr Gln Glu Arg Asn Arg Val Phe Ala	
55 60 65	
tca ggc caa ctt ctg aag ttt cta cca gct gaa gtt gct gat tct ggt	295
Ser Gly Gln Leu Leu Lys Phe Leu Pro Ala Glu Val Ala Asp Ser Gly	
70 75 80	
att tat acc tgt att gtc aga agt ccc aca ttc aat agg act gga tat	343
Ile Tyr Thr Cys Ile Val Arg Ser Pro Thr Phe Asn Arg Thr Gly Tyr	
85 90 95	
gcg aat gtc acc ata tat aaa aaa caa tca gat tgc aat gtt cca gat	391
Ala Asn Val Thr Ile Tyr Lys Lys Gln Ser Asp Cys Asn Val Pro Asp	
100 105 110 115	
tat ttg atg tat tca aca gta tct gga tca gaa aaa aat tcc aaa att	439
Tyr Leu Met Tyr Ser Thr Val Ser Gly Ser Glu Lys Asn Ser Lys Ile	
120 125 130	
tat tgt cct acc att gac ctc tac aac tgg aca gca cct ctt gag tgg	487
Tyr Cys Pro Thr Ile Asp Leu Tyr Asn Trp Thr Ala Pro Leu Glu Trp	
135 140 145	
ttt aag aat tgt cag gct ctt caa gga tca agg tac agg gcg cac aag	535
Phe Lys Asn Cys Gln Ala Leu Gln Gly Ser Arg Tyr Arg Ala His Lys	
150 155 160	
tca ttt ttg gtc att gat aat gtg atg act gag gac gca ggt gat tac	583
Ser Phe Leu Val Ile Asp Asn Val Met Thr Glu Asp Ala Gly Asp Tyr	
165 170 175	
acc tgt aaa ttt ata cac aat gaa aat gga gcc aat tat agt gtg acg	631
Thr Cys Lys Phe Ile His Asn Glu Asn Gly Ala Asn Tyr Ser Val Thr	
180 185 190 195	
gcg acc agg tcc ttc acg gtc aag gat gag caa ggc ttt tct ctg ttt	679
Ala Thr Arg Ser Phe Thr Val Lys Asp Glu Gln Gly Phe Ser Leu Phe	
200 205 210	
cca gta atc gga gcc cct gca caa aat gaa ata aag gaa gtg gaa att	727
Pro Val Ile Gly Ala Pro Ala Gln Asn Glu Ile Lys Glu Val Glu Ile	
215 220 225	

gga aaa aac gca aac cta act tgc tct gct tgt ttt gga aaa ggc act 775
 Gly Lys Asn Ala Asn Leu Thr Cys Ser Ala Cys Phe Gly Lys Gly Thr
 230 235 240

cag ttc ttg gct gcc gtc ctg tgg cag ctt aat gga aca aaa att aca 823
 Gln Phe Leu Ala Ala Val Leu Trp Gln Leu Asn Gly Thr Lys Ile Thr
 245 250 255

gac ttt ggt gaa cca aga att caa caa gag gaa ggg caa aat caa agt 871
 Asp Phe Gly Glu Pro Arg Ile Gln Gln Glu Gly Gln Asn Gln Ser
 260 265 270 275

ttc agc aat ggg ctg gct tgt cta gac atg gtt tta aga ata gct gac 919
 Phe Ser Asn Gly Leu Ala Cys Leu Asp Met Val Leu Arg Ile Ala Asp
 280 285 290

gtg aag gaa gag gat tta ttg ctg cag tac gac tgt ctg gcc ctg aat 967
 Val Lys Glu Glu Asp Leu Leu Leu Gln Tyr Asp Cys Leu Ala Leu Asn
 295 300 305

ttg cat ggc ttg aga agg cac acc gta aga cta agt agg aaa aat cca 1015
 Leu His Gly Leu Arg Arg His Thr Val Arg Leu Ser Arg Lys Asn Pro
 310 315 320

agt aag gag tgt ttc tgagactttg atcacctgaa ctttctctag caagtgtgag 1070
 Ser Lys Glu Cys Phe
 325

cagaatggag tgtggttcca agagatccat caagacaatg ggaatggcct gtgccataaa 1130
 atgtgcttct cttcttcggg atgttggttg ctgtctgac tttgtagact gttcctgttt 1190
 gctgggagct tctctgctgc ttaaattggt cgctctcccc cactccctcc tategttggt 1250
 ttgtctagaa cactcagctg cttctttggt catccttggt ttctaacttt atgaactccc 1310
 tctgtgtcac tgtatgtgaa aggaaatgca ccaacaaccg aaaactg 1357

<210> 45
 <211> 328
 <212> PRT
 <213> Homo sapiens

<400> 45
 Met Gly Phe Trp Ile Leu Ala Ile Leu Thr Ile Leu Met Tyr Ser Thr
 1 5 10 15
 Ala Ala Lys Phe Ser Lys Gln Ser Trp Gly Leu Glu Asn Glu Ala Leu
 20 25 30
 Ile Val Arg Cys Pro Arg Gln Gly Lys Pro Ser Tyr Thr Val Asp Trp
 35 40 45
 Tyr Tyr Ser Gln Thr Asn Lys Ser Ile Pro Thr Gln Glu Arg Asn Arg
 50 55 60

Val Phe Ala Ser Gly Gln Leu Leu Lys Phe Leu Pro Ala Glu Val Ala
 65 70 75 80
 Asp Ser Gly Ile Tyr Thr Cys Ile Val Arg Ser Pro Thr Phe Asn Arg
 85 90 95
 Thr Gly Tyr Ala Asn Val Thr Ile Tyr Lys Lys Gln Ser Asp Cys Asn
 100 105 110
 Val Pro Asp Tyr Leu Met Tyr Ser Thr Val Ser Gly Ser Glu Lys Asn
 115 120 125
 Ser Lys Ile Tyr Cys Pro Thr Ile Asp Leu Tyr Asn Trp Thr Ala Pro
 130 135 140
 Leu Glu Trp Phe Lys Asn Cys Gln Ala Leu Gln Gly Ser Arg Tyr Arg
 145 150 155 160
 Ala His Lys Ser Phe Leu Val Ile Asp Asn Val Met Thr Glu Asp Ala
 165 170 175
 Gly Asp Tyr Thr Cys Lys Phe Ile His Asn Glu Asn Gly Ala Asn Tyr
 180 185 190
 Ser Val Thr Ala Thr Arg Ser Phe Thr Val Lys Asp Glu Gln Gly Phe
 195 200 205
 Ser Leu Phe Pro Val Ile Gly Ala Pro Ala Gln Asn Glu Ile Lys Glu
 210 215 220
 Val Glu Ile Gly Lys Asn Ala Asn Leu Thr Cys Ser Ala Cys Phe Gly
 225 230 235 240
 Lys Gly Thr Gln Phe Leu Ala Ala Val Leu Trp Gln Leu Asn Gly Thr
 245 250 255
 Lys Ile Thr Asp Phe Gly Glu Pro Arg Ile Gln Gln Glu Glu Gly Gln
 260 265 270
 Asn Gln Ser Phe Ser Asn Gly Leu Ala Cys Leu Asp Met Val Leu Arg
 275 280 285
 Ile Ala Asp Val Lys Glu Glu Asp Leu Leu Leu Gln Tyr Asp Cys Leu
 290 295 300
 Ala Leu Asn Leu His Gly Leu Arg Arg His Thr Val Arg Leu Ser Arg
 305 310 315 320
 Lys Asn Pro Ser Lys Glu Cys Phe
 325

<210> 46

<211> 72

<212> DNA

<213> Mus musculus

<400> 46

ttattgctaa tgtttatcaa tgtcttggtg atagtcttaa aagtgttctg gattgagggt 60
gctctgttct gg 72

<210> 47
<211> 1011
<212> DNA
<213> Mus musculus

<400> 47
atgattgaca gacagagaat gggacttttg gctttggcaa ttctgacact tcccatgtat 60
ttgacagtta cggagggcag taaatcgccc tggggtcttg aaaatgaggc tttaattgtg 120
agatgcccc aaagaggacg ctcgacttat cctgtggaat ggtattactc agatacaaat 180
gaaagtattc ctactcaaaa aagaaatcgg atctttgtct caagagatcg tctgaagttt 240
ctaccagcca gagtcgaaga ctctgggatt tatgcttggt ttatcagaag cccaacttg 300
aataagactg gatacttgaa tgtcaccata cataaaaagc cgccaagctg caatatccct 360
gattatttga tgtactcgac agtacgtgga tcagataaaa atttcaagat aagctgtcca 420
acaattgacc tgtataattg gacagcacct gttcagtggt ttaagaactg caaagctctc 480
caagagccaa gggtcagggc acacaggtcc tacttggtca ttgacaacgt gactcatgat 540
gatgaagggtg actacacttg tcaattcaca cacgcggaga atggaaccaa ctacatcgtg 600
acggccacca gatcattcac agttgaagaa aaaggctttt ctatgtttcc agtaattaca 660
aatcctccat acaaccacac aatggaagtg gaaataggaa aaccagcaag tattgcctgt 720
tcagcttgct ttggcaaagg ctctcacttc ttggctgatg tcctgtggca gattaacaaa 780
acagtagttg gaaatttttg tgaagcaaga attcaagaag aggaaggctg aaatgaaagt 840
tccagcaatg acatggattg tttaacctca gtgttaagga taactgggtg gacagaaaag 900
gacctgtccc tggaatatga ctgtctggcc ctgaaccttc atggcatgat aaggcacacc 960
ataaggctga gaaggaaaca accaagtaag gagtgtccct cacacattgc t 1011

<210> 48
<211> 337
<212> PRT
<213> Mus musculus

<400> 48
Met Ile Asp Arg Gln Arg Met Gly Leu Trp Ala Leu Ala Ile Leu Thr
1 5 10 15
Leu Pro Met Tyr Leu Thr Val Thr Glu Gly Ser Lys Ser Ser Trp Gly
20 25 30

Leu Glu Asn Glu Ala Leu Ile Val Arg Cys Pro Gln Arg Gly Arg Ser
 35 40 45
 Thr Tyr Pro Val Glu Trp Tyr Tyr Ser Asp Thr Asn Glu Ser Ile Pro
 50 55 60
 Thr Gln Lys Arg Asn Arg Ile Phe Val Ser Arg Asp Arg Leu Lys Phe
 65 70 75 80
 Leu Pro Ala Arg Val Glu Asp Ser Gly Ile Tyr Ala Cys Val Ile Arg
 85 90 95
 Ser Pro Asn Leu Asn Lys Thr Gly Tyr Leu Asn Val Thr Ile His Lys
 100 105 110
 Lys Pro Pro Ser Cys Asn Ile Pro Asp Tyr Leu Met Tyr Ser Thr Val
 115 120 125
 Arg Gly Ser Asp Lys Asn Phe Lys Ile Thr Cys Pro Thr Ile Asp Leu
 130 135 140
 Tyr Asn Trp Thr Ala Pro Val Gln Trp Phe Lys Asn Cys Lys Ala Leu
 145 150 155 160
 Gln Glu Pro Arg Phe Arg Ala His Arg Ser Tyr Leu Phe Ile Asp Asn
 165 170 175
 Val Thr His Asp Asp Glu Gly Asp Tyr Thr Cys Gln Phe Thr His Ala
 180 185 190
 Glu Asn Gly Thr Asn Tyr Ile Val Thr Ala Thr Arg Ser Phe Thr Val
 195 200 205
 Glu Glu Lys Gly Phe Ser Met Phe Pro Val Ile Thr Asn Pro Pro Tyr
 210 215 220
 Asn His Thr Met Glu Val Glu Ile Gly Lys Pro Ala Ser Ile Ala Cys
 225 230 235 240
 Ser Ala Cys Phe Gly Lys Gly Ser His Phe Leu Ala Asp Val Leu Trp
 245 250 255
 Gln Ile Asn Lys Thr Val Val Gly Asn Phe Gly Glu Ala Arg Ile Gln
 260 265 270
 Glu Glu Glu Gly Arg Asn Glu Ser Ser Ser Asn Asp Met Asp Cys Leu
 275 280 285
 Thr Ser Val Leu Arg Ile Thr Gly Val Thr Glu Lys Asp Leu Ser Leu
 290 295 300
 Glu Tyr Asp Cys Leu Ala Leu Asn Leu His Gly Met Ile Arg His Thr
 305 310 315 320
 Ile Arg Leu Arg Arg Lys Gln Pro Ser Lys Glu Cys Pro Ser His Ile
 325 330 335

Ala

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING.

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1, 4-11, 13, and 14, drawn to first method of treatment of ischemic disorder by giving a 200 gene product.

Group II, claims 2, 4-12, 15, and 16, drawn to second method of treatment of ischemic disorder by gene therapy.

Group III, claims 3-11 and 17-21, drawn to third method of treatment of ischemic disorder by giving an antibody.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: each invention is drawn to a separate inventive concept. Invention I is drawn to a gene product, i.e., proteins. Invention II is drawn to gene therapy, i.e., administration of DNA. Invention III is drawn to passive immunity, i.e., administration of antibody.